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THE PREFERENCES OF HONEYBEES FOR SOLUTIONS OF VARIOUS SUGARS WHICH OCCUR IN NECTAR

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(*Received* 15 *December* 1951)

INTRODUCTION

When foraging for nectar from plants which flower at the same period honeybees appear to show decided preferences for some species, whereas they do not visit others. Several workers, including Vansell (1934), Kleber (1935) and Butler (1945), found that the attractiveness of a source of nectar was dependent on the amount available and the concentration of sugar in it. According to von Frisch (1946*a*, *b*) an adequate supply of nectar of sufficiently high concentration is necessary to induce nectar-gathering bees to dance on returning to the hive, and thus to indicate the position of a particular nectar crop to other members of the colony. Von Frisch (1934) showed that solutions of fructose, glucose and sucrose, the normal constituents of nectar, were readily accepted by honeybees, while Phillips (1927) and Vogel (1931) found that these sugars were of nutritive value to bees and could be metabolized by them.

No detailed experimental work appears to have been carried out, however, to determine whether the variations in the sugar composition of different nectars, which have been demonstrated by von Planta (1886), Beutler (1930), Vansell (1944), Wykes (1952) and others, may be a contributory factor influencing the selection of certain nectars by bees.

The observations of Kunze (1927, 1933) and von Frisch (1934) on the responses of bees towards various solutions were based on experiments designed mainly to establish threshold values, and their data do not agree. A series of experiments was therefore carried out to determine directly whether honeybees, when offered a choice of different sugars which occur in nectar, discriminate between them.

EXPERIMENTAL METHODS

Equal volumes of sugar solutions of different composition, but of the same total sugar concentration, were made available to bees under both field and laboratory conditions. Since Will (1885), Kunze (1927) and von Frisch (1934) had shown that the taste sensitivity of individual honeybees is subject to wide variation, a large number of bees was used in each experiment.

Sugar solutions

Single solutions or mixtures of sucrose, glucose and fructose were used in the field experiments, while in the laboratory series maltose, which may occur in nectar from some species, was also included. The solutions offered in each experiment were

freshly made up with distilled water from analytical grade sugars, when available. As it was not possible to obtain an A.R. sample of fructose in sufficient quantity, the 'laboratory chemical' product of the firm of Hopkin and Williams was used.

A. *Field experiments*

Foragers from a honeybee colony were first trained to visit a table placed in a field approximately one-eighth of a mile from an apiary, and a constant stream of syrup-collecting bees was maintained throughout the period of the experiments during July and August 1949.

100 ml. of each solution to be tested were measured into Petri dishes (10 cm. diameter) placed on a square board serving as the top of the table, the board being painted with white enamel to prevent undue heating of the solutions. The test solutions were arranged in either a 5×5 or 6×6 Latin square, and a different random arrangement was used for each experiment. To allow the bees to alight easily and drink from the dishes a disk of thin Balsa wood was placed on the surface of each solution. The number of bees drinking from each dish was counted at 10 min. intervals over a period of 3 or 4 hr. In order to prevent possible recognition of dishes by their position on the table, the top of the table was turned through 90° at intervals. It was also frequently cleared of bees, since it has been suggested that they may be attracted to a site by observing there the presence of other bees. In order that bees approaching the table would not be attracted by the scent possibly remaining from other bees which had previously visited the dishes, the solutions, Balsa wood disks, and dishes were renewed at intervals. After the first exploratory experiment distilled water was included in each experiment as a control measure to allow for possible acceptance of a solution due merely to thirst, and not due to preference for the solutes.

In the first two experimental series, solutions of concentration 17·10 g./100 ml. were used. However, in the two series following, when the weather was warmer and the number of flying bees appeared to have increased greatly, solutions of concentration 8·55 g./100 ml. were used in order to reduce the number of bees visiting the dishes, and thus minimize the possible error involved in taking counts. Even though this concentration level was below the normal threshold of acceptance for bees, large numbers continued to come to the dishes, suggesting that the naturally occurring nectar sources available in the area were poor. Each series of experiments was carried out on warm, clear days for some period between 14 and 18 hr. (B.S.T.).

B. *Laboratory experiments*

The exploratory field work suggested that additional useful information could be obtained by further experiments. During winter 1949–50 an additional series was carried out, in which the same set of solutions, at different concentration levels in the different experiments, was made available to bees under laboratory conditions. The concentration range of the solutions offered, consisting either of single sugars

or mixtures of sugars in equal proportions by weight, varied regularly (increases of 8·55 g./100 ml.) from 17·10 to 51·30 g./100 ml.

Preferences shown by the bees for these solutions were determined by a method and apparatus similar to that used by Butler, Finney & Schiele (1943) when working on poison trials. Equal volumes of each sugar solution were delivered from a microburette into individual cells on one side of a piece of brood comb, approximately 14 × 14 cm., placed horizontally. In order to reduce possible edge effects the experimental solutions, which were set out in the form of an 8 × 8 or 9 × 9 Latin square with a different random arrangement for each experiment, were surrounded by a number of cells containing sucrose solution of the same concentration. No correction factor was applied for loss of volume by evaporation during the experimental period, as this was assumed to be approximately constant for each series, the solutions being of equal concentration. The piece of brood comb was placed inside a glass 'arena' of 31 cm. internal diameter, the upper chamber being 22 cm. in height. A total of 100–200 bees, withdrawn at random from the same hive colony, were introduced into the arena and allowed to feed from the sugar solutions for an interval of 1 or 2 hr., depending on the rate of feeding. During this time the cells were kept under observation to ensure that none became completely empty.

On certain days it was found that the bees would not feed readily, for no understandable reason, and the experiment had to be abandoned. Usually, however, the bees became distributed evenly on the surface of the comb, wandered over it, and appeared to select and reject certain solutions in the different cells. After the withdrawal of the bees from the arena the amount of solution remaining in each experimental cell was measured with a specially adapted micropipette, calibrated accurately in arbitrary units.

The conditions for the different experiments were not necessarily uniform as certain factors were quite obviously subject to variation. The rate of uptake of sugar solutions was found by Betts (1929) to be largely independent of viscosity, up to concentrations of 50% which includes the range offered in these experiments. Von Frisch (1934) found that the acceptance of sugar solutions by bees was not influenced by temperature, osmotic pressure, nor viscosity of the solutions. However, variables such as the concentration of the solutions and physiological state of the bees, which were shown by the work of von Frisch to influence the acceptance of sugar solutions, may be regarded as constant for any one experiment in this series. The preferences shown by bees for each sugar solution in any individual experiment may be assessed from (*a*) number of bee visits to each solution in the field experiments, (*b*) uptake of each solution in the laboratory experiments.

RESULTS

Field experiments

The observed number of bee visits to the different solutions are summarized in Table 1. For Exps. 2, 3 and 4, in which relatively few visits were made to dishes containing distilled water compared with those containing sugar solutions, the

number of visits to water (not exceeding five in any experiment) was omitted from analyses of results.

Table 1. *Acceptance of sugar solutions in field experiments*

No. of exp.	Conc. soln. (g./100 ml.)	Mean no. of visits/solution					S.E.
		F	G	S	SGF 1:1:1		
1	17·10	44	106	128	162		7·4
		F	G	S	SGF 2:1:1	SGF 1:1:1	
2	17·10	78	141	152	154	190	8·7
		S	GF 1:1	SF 1:1	SG 1:1	SGF 1:1:1	
3	8·55	130	92	95	119	152	8·9
		S	SGF 1:2:1	SGF 1:1:2	SGF 2:1:1	SGF 1:1:1	
4	8·55	195	168	171	194	248	17·3

In all tables F = fructose, G = glucose, S = sucrose, M = maltose.

The data in Table 1 show differences in preference for the sugar solutions in each experiment. The acceptance of both sucrose and glucose relative to fructose was markedly high. The preferences for sucrose appeared to be higher than for glucose, but the differences were not significant (although $P = 0{\cdot}05$ in Exp. 1; in Exp. 2, $P = 0{\cdot}5$). Consistent preferences were shown in each experiment for the mixture containing equal proportions of sucrose, glucose and fructose, compared with solutions of single sugars, mixtures of two sugars, or the same sugars available in different proportions (P never $> 0{\cdot}05$).

Laboratory experiments

The summarized results for the uptake of different sugar solutions are given in Table 2.

Table 2. *Acceptance of sugar solutions in laboratory experiments*

No. of exp.	Conc. soln. (g./100 ml.)	Mean uptake soln. (arbitrary units)								S.E.
		F	M	G	S	SG	SGM	SGF	SGFM	
1	17·10	24·8	26·3	30·8	34·1	33·2	34·2	47·6	23·9	4·33
2*a*	25·65	42·3	52·4	79·1	90·1	82·6	77·5	87·6	55·0	5·53
2*b*	25·65	60·0	70·2	83·7	93·5	83·4	89·7	95·6	70·5	5·04
3	34·20	38·6	47·1	54·6	91·0	64·0	58·0	86·4	50·3	6·29
4	42·75	81·7	98·2	117·9	126·5	119·7	112·5	121·4	101·8	4·54
5	51·30	48·3	56·5	91·2	103·9	97·5	81·8	100·7	68·8	6·06

From these results it may be seen that at each concentration the solutions of single sugars were accepted in the following descending order of preference: sucrose, glucose, maltose, fructose.

Since the standard errors shown in Table 2 did not vary greatly in the different experiments, an analysis of the combined results was carried out in order to deter-

mine whether concentration influenced the relative acceptance of the solutions. This suggested that the relative preferences for the solutions were not the same at all concentration levels. A real difference was found to occur between Exp. 1 and the rest of the series. However, the existing data provide no evidence for change in relative preference with changing concentration in Exps. 2–5.

In order to examine in greater detail the acceptance of mixtures, the observed results for uptake of solutions of mixed sugars (Table 2) were compared with the calculated mean uptake of solutions of their constituent sugars, as shown in Table 3.

Table 3. *Acceptance of mixtures compared with single sugars in laboratory experiments*

Mixture	Determination	Mean uptake (arbitrary units)					
		Exp. 1	Exp. 2*a*	Exp. 2*b*	Exp. 3	Exp. 4	Exp. 5
SG	SG actual	33·2	82·6	83·4	64·0	119·7	97·5
	½ (S+G)	32·4	84·6	88·6	72·8	122·2	97·6
	Difference	0·8	−2·0	−5·2	−8·8	−2·5	−0·1
	S.E. difference	5·30	6·77	6·17	7·70	5·56	7·42
	P	0·9	0·8	0·4	0·3	0·7	>0·9
SGM	SGM actual	34·2	77·5	89·6	58·0	112·5	81·8
	⅓ (S+G+M)	30·4	73·9	82·5	64·2	114·2	83·9
	Difference	3·8	3·6	7·1	−6·2	−1·7	−2·1
	S.E. difference	5·00	6·39	5·82	7·26	5·24	7·00
	P	0·5	0·6	0·2	0·4	0·7	0·8
SGF	SGF actual	47·6	87·6	95·6	86·4	121·4	100·7
	⅓ (S+G+F)	29·9	70·5	79·1	61·4	108·7	81·1
	Difference	17·9	17·1	16·5	25·0	12·7	19·6
	S.E. difference	5·00	6·39	5·82	7·26	5·24	7·00
	P	0·01	0·02	0·02	0·01	0·05	0·01
SGFM	SGFM actual	23·9	55·0	70·5	50·3	101·8	6·88
	¼ (S+G+F+M)	29·0	66·0	76·9	57·8	106·1	75·0
	Difference	−5·1	−11·0	−6·4	−7·5	−4·3	−6·2
	S.E. difference	4·84	6·18	5·63	7·03	5·08	6·78
	P	0·3	0·1	0·4	0·3	0·3	0·3
	t, 5 % level	8·75	11·18	10·19	12·71	9·18	12·25
	t, 1 % level	11·71	14·95	13·63	17·01	12·28	16·39

Table 3 shows that at all the concentrations offered the uptake of the sucrose-glucose-fructose mixture was significantly greater than the mean uptake of the constituent sugars. At no concentration was the uptake of the sucrose-glucose mixture or of the sucrose-glucose-maltose mixture significantly different from the mean uptake of the constituent sugars. This applies equally to the sucrose-glucose-fructose-maltose mixture, but in this case, if the results for all concentrations are combined, the uptake of the mixture is found to be significantly lower than the mean uptake of the constituent sugars.

DISCUSSION

It was realized that the environmental conditions under which experiments in the laboratory were carried out differed widely from the normal foraging conditions for honeybees. The possibility of reaching incorrect conclusions by employing such

methods when attempting to interpret insect response has been emphasized by von Frisch (1919), Marshall (1935) and others. However, the results of the initial experiment attempted in the arena (Table 2, no. 1) agreed with those of the experiments at the same concentration (17·1 g./100 ml.) carried out in the field, which approximated to normal conditions with choice of naturally occurring crops. Subsequent laboratory experiments also provided further confirmatory evidence. Thus it was assumed that the results of the arena experiments might be accepted as a reliable index of the relative attractiveness of the different sugar solutions to bees.

According to von Frisch (1934) different sugars have only one quality of taste stimulus, that of sweetness, to bees. Skramlik (1926) suggested that the existence of only one quality of sweet taste appears to be of general occurrence throughout the animal kingdom, but he found that different substances may vary in the intensity of sweet taste produced. Studies of the comparative physiology of taste show that the response to different sugars appears to be a specific characteristic, the order of relative preference varying according to the species.

Single sugars

The results for relative acceptance of solutions of single sugars offered in these field and laboratory experiments do not agree with those of earlier investigators. Von Frisch (1934) concluded, from observations of bee visits to dishes containing sugar solutions which were made available at different times and not offered simultaneously, that glucose and fructose were equally attractive to bees, and that sucrose was the most attractive sugar. The results of Kunze (1927, 1933) on comparison of the length of time taken by bees to empty syrup-containing dishes, and on 'all or none' proboscis extension reactions to different sugars, were extremely variable, and the conclusions appear to be based on somewhat inadequate data.

Considering the apparent order of preference suggested by the results of the present experiments, it is interesting to examine the hypothesis of Vogel (1931) that the only indication of the value of a carbohydrate food substance to bees is the sweet taste of the particular substance. However, since Vogel found that fructose was of greater nutritive value to adult honeybees than glucose, sucrose having an intermediate value, it can be seen that this hypothesis, although it may apply in general, cannot be accepted on a quantitative basis for the selection of solutions of single sugars by bees.

Mixed sugars

From experiments in which solutions of mixed sugars were offered to bees von Frisch (1934) suggested that the physiological effects of more than one sugar on taste perception would be additive. The results presented here, however, show that the observed preferences for solutions of mixed sugars may differ from those which would be predicted on the basis of a purely additive effect of their constituent sugars. In wider studies of the relative sweetness to man of mixtures of sugars compared with the sum of the sweetness of the individual components, apparent discrepancies were observed also by Pauli (1921), Paul (1925), Cameron (1947) and

others. Dahlberg & Penczek (1941) attempted to explain this 'supplemental action' on a physiological basis, suggesting that the rate of perception of maximum sweetness of the component sugars in a mixture is not necessarily equal; that the maximum intensity of the stimulus of the second sugar may be reached more slowly, and thus the effect of this sugar supplements that of the first. However, this hypothesis does not explain the apparently inconsistent responses of bees to solutions of mixed sugars offered in the laboratory experiments. Sucrose-glucose and sucrose-glucose-maltose mixtures appeared to give rise to no synergic effect. Although the acceptance of the sucrose-glucose-fructose mixture was markedly higher than that calculated on an additive basis, the inclusion of maltose in this mixture appeared to lead to acceptance somewhat lower than the mean acceptance of the four constituent sugars.

Effect of concentration

By comparative taste experiments it has been shown that the relative sweetness to man and other animals of sucrose, glucose and fructose varies with varying concentration.

Dahlberg & Penczek (1941) attempted to explain the decreasing sensation produced with increasing concentration of the sapid substance on the basis of sense saturation, suggesting that the relative efficiency of each molecule falls off in more concentrated solutions. Subsequent experiments of Cameron (1947) did not fully support this explanation. Kunze (1933) had observed earlier that the response of bees to different sugar solutions varied at different concentrations. Insufficient data are available from the present investigation to determine conclusively how the relative acceptances of sugar solutions offered to bees may vary with varying concentrations. The relative uptake at the normal threshold concentration (17·1 g./100 ml.) appeared to differ from that for the higher levels of concentration offered in the laboratory experiments, which corresponded to the range of most nectars available in the field. It is interesting to note that Cameron (1947) emphasized that threshold values may be unreliable for quantitative comparisons of the relative sweetness of different sugars to man.

Although the present knowledge of the processes which give rise to taste stimulation is slight, it may be assumed that the sensation of taste produced is some function of the molecules of the stimulating sapid substance (Cohn, 1916). No direct relationship seems to exist, however, between the chemical constitution of sugars and the relative response observed in different animals (Täufel, 1925; von Frisch, 1934; Cameron, 1947). An examination of the results of field and laboratory experiments together with the configurational formulae of the four sugars offered reveals no apparent relationship between the chemical constitution of the sugars and their relative attractiveness to bees. In the absence of further knowledge regarding the exact nature of the physiological processes involved in the stimulation of the gustatory organs and leading to perception of taste, no theories appear to exist which explain adequately the observed differences in preferences for the sugars. The possibility that molecular groups critical for taste stimulus may become

physiologically active only in certain forms of construction, or in combination with other substances not yet determined, has been pointed out by von Frisch (1934) and others. It is possible that some aspect of the property of biological specificity may be involved. Advances in the general understanding of the physico-chemical basis for specificity of activity exhibited in various biological systems may provide an acceptable interpretation of such reactions. This possibility should be considered in relation to the apparent inconsistencies in the preferences for solutions of mixed sugars compared with their constituent sugars in single solution. It may be suggested tentatively that a mixture such as sucrose-glucose-fructose, for example, may constitute a particular molecular complex necessary to produce the maximum effective stimulus of the perceptory areas resulting in the relatively high acceptance of this mixture. Since nectar, the naturally occurring source of carbohydrate for honeybees, consists of these three sugars, this apparently anomalous high degree of relative preference for the sucrose-glucose-fructose mixture may be of some biological significance. It is possible that bees may exhibit some inherent preference for, or that they may be conditioned physiologically to, such a solution as a result of repeated acceptance and utilization of nectar in the field and honey within the hive; but no evidence supporting such tentative hypotheses is provided by this investigation.

These observations suggest that the constituent sugars may play a part in determining the relative attractiveness of different nectars to bees. It appears therefore that the visits of honeybees to flowers are not only influenced by the volume and sugar concentration of nectar, but may also be influenced by the individual sugars of which it is composed.

SUMMARY

1. In field and in laboratory experiments bees were offered equal volumes of sugar solutions, of different composition but the same total concentration. It was found that sugars which occur in nectar were not equally attractive to bees.

2. Consistent preferences were shown for solutions of single sugars in the following descending order: sucrose, glucose, maltose, fructose.

3. The acceptances of some mixtures differed from those predicted on the basis of an additive effect of the constituent sugars. Anomalous high preferences were shown for sucrose-glucose-fructose solutions.

4. The concentration of the solution appeared to influence the observed preferences, the relative acceptances of solutions at normal threshold level differing from those at higher levels in the series of laboratory experiments.

5. No direct relationship appeared to exist between the chemical constitution of the sugars offered and their acceptance by bees; and no evidence was found to explain the observed differences in preferences for solutions of either single or mixed sugars.

6. Attention is drawn to the possible biological significance of such selective responses by bees—it appears that the sugar composition of nectar may be a contributory factor influencing the visits of bees to flowers.

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REFERENCES

Betts, A. D. (1929). Das Aufnahmevermögen der Bienen beim Zuckerwasserfüttern. *Arch. Bienenk.* **10**, 29–37.

Beutler, R. (1930). Biologisch-chemische Untersuchungen am Nektar von Immenblumen. *Z. vergl. Physiol.* **12**, 72–176.

Butler, C. G. (1945). Influence of various physical and biological factors of the environment on honeybee activity. An examination of the relationship between activity and nectar concentration and abundance. *J. Exp. Biol.* **21**, 5–12.

Butler, C. G., Finney, D. J. & Schiele, P. (1943). Experiments on the poisoning of honeybees in insecticidal and fungicidal sprays used in orchards. *Ann. Appl. Biol.* **30**, 143–50.

Cameron, A. T. (1947). The taste sense and the relative sweetness of sugars and other sweet substances. *Sug. Res. Foundat. Inc. N.Y. Sci. Rep. Ser.* no. 9.

Cohn, G. (1916). Geschmack und Konstitution bei organischen Verbindungen. *Samml. chem. Vortr.* **22**, 1–100.

Dahlberg, A. C. & Penczek, E. S. (1941). The relative sweetness of sugars as affected by concentration. *Tech. Bull. N.Y. St. Agric. Exp. Sta.* no. 258.

Frisch, K. von (1919). Über den Geruchsinn der Bienen und seine blütenbiologische Bedeutung. *Zool. Jb. Physiol.* **37**, 1–238.

Frisch, K. von (1934). Über den Geschmackssinn der Bienen. Ein Beitrag zur vergleichenden Physiologie des Geschmacks. *Z. vergl. Physiol.* **21**, 1–156.

Frisch, K. von (1946*a*). Die 'Sprache' der Bienen und ihre Nutzanwendung in der Landwirtschaft. *Experientia*, **2**, 1–21.

Frisch, K. von. (1946*b*). Die Tänze der Bienen. *Öst. Zool. Z.* **1**, 1–48.

Kleber, E. (1935). Hat das Zeitgedächtnis der Bienen biologische Bedeutung? *Z. vergl. Physiol.* **22**, 221–62.

Kunze, G. (1927). Einige Versuche über den Geschmackssinn der Honigbiene. *Zool. Jb.* (Abt. allg. Zool. Phys.), **44**, 287–314.

Kunze, G. (1933). Einige Versuche über den Antennengeschmackssinn der Honigbiene. *Zool. Jb.* (Abt. allg. Zool. Phys.), **52**, 465–512.

Marshall, J. (1935). The location of olfactory receptors in insects; a review of experimental evidence. *Trans. R. ent. Soc. Lond.* **83**, 49–72.

Paul, T. (1925). Physikalische Chemie der Lebensmittel. 5. Der Süssgrad der Süsstoffe. *Z. Elektrochem.* **27**, 539–46.

Pauli, R. (1921). Über die Messung der Süsskraft von künstlichen Süsstoffen. *Biochem. Z.* **125**, 97–105.

Phillips, E. F. (1927). The utilisation of carbohydrates by honeybees. *J. Agric. Res.* **35**, 385–428.

Planta, A. von (1886). Über die Zusammensetzung einiger Nektar-Arten. *Hoppe-Seyl. Z.* **10**, 227–47.

Skramlik, E. von (1926). Physiologie des Geschmackssinnes. *Handb. normalen Path. Physiol.* **11**, 306–92.

Täufel, K. (1925). Studien über die Beziehungen zwischen dem chemischen Aufbau und dem Geschmack süssschmeckender Stoffe. *Biochem. Z.* **165**, 96–101.

Vansell, G. H. (1934). Relation between the nectar concentration in fruit blossoms and the visits of honeybees. *J. Econ. Ent.* **27**, 943–5.

Vansell, G. H. (1944). Some western nectars and their corresponding honeys. *J. Econ. Ent.* **37**, 530–3.

Vogel, B. (1931). Über die Beziehungen zwischen Süssgeschmack und Nährwert von Zuckern und Zuckeralkoholen bei der Honigbiene. *Z. vergl. Physiol.* **14**, 273–347.

Will, F. (1885). Das Geschmacksorgan der Insekten. *Z. wiss. Zool.* **42**, 674–707.

Wykes, G. R. (1952). An investigation of the sugars present in the nectar of flowers of various species. *New Phytol.* **51**, 210.

FLOW ORIENTATION AS A POSSIBLE EXPLANATION OF 'WAVE-MOTION' AND 'RHEOTAXIS' OF SPERMATOZOA

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(*Received* 25 *July* 1951)

(With Six Text-figures)

INTRODUCTION

'Wave-motion' may have been observed by some of the pioneer seminologists. Blumenbach in 1792, describing the work of Leeuwenhoek, Hooke and others, writes: 'The spermatozoa were discovered in the semen of all animals;...in the semen of the ram they beheld them moving forward in a troop with great gravity like a flock of sheep' (Cole, 1930). This description is amusingly animalculistic, since it implies the existence in the spermatozoa of a pattern of behaviour exhibited by the grown animal, but it is true that in the naturally concentrated semen of the ram and bull, and of other animals when the semen is concentrated by centrifugation, the spermatozoa do not move at random in all directions independently of each other but in waves. If a fairly thick layer of semen is examined with the low power of the microscope, the interplay of these waves can be observed as apparent differences of opacity, and light and dark waves form and disappear and swirl over the field of focus. Descriptions and photographs of this phenomenon have appeared in several papers (Laing, 1945; Blom, 1946; Rothschild, 1949*a*, *b*). The phenomenon can also be observed with the naked eye in normal incident light on the surface of a drop of semen or through the wall of a glass container. The surface presents a mottled appearance, and the mottles are continuously changing their shape and direction of movement.

Another early discovery was that mammalian spermatozoa are orientated in the flow of a suspending medium and swim against the current (Lott, 1872; Hensen, 1876; Kraft, 1890; Roth, 1904; Adolphi, 1905). Adolphi came to the conclusion that the rate of movement of the spermatozoa relative to the velocity of flow of the current was independent of the velocity of the current. But Yamane & Ito (1932) claim that with horse spermatozoa the velocity of movement relative to the flow increases with the velocity of flow up to a maximum of about 200 μ per sec., which was about twice the velocity of the spermatozoa in still medium. This maximum was reached when the velocity of the counter current was approximately equal to that of the spermatozoa in still medium (86 μ/sec.). This last paper suggests therefore that 'rheotaxis' is not merely a phenomenon of orientation, but that the counter current stimulates the spermatozoa to increased activity. No collateral evidence was, however, produced to show that either the energy output or the efficiency of movement were altered by the opposing current, and the results are open to an alternative explanation (see Discussion, p. 529).

Neither 'wave-motion' nor 'rheotaxis' have been explained in terms of established physical principles. Nevertheless, they have this in common that both entail a certain degree of orientation of the spermatozoa or an orderly departure from a completely random distribution of the spermatozoa in the suspension. In this paper an attempt is made to show that both may depend upon the flow orientation of elongated particles in suspension, a phenomenon which is well known to the physicist and capable of complete solution for inert particles.

EXPERIMENTAL

Bull semen was used throughout the experiments, except when other material is specified. Only dense samples (700–1500 × 10/ml.) showing active 'wave-motion' were used. The first point was to determine whether spermatozoa are orientated by flow. This might almost be taken for granted, and as a matter of simple observation,

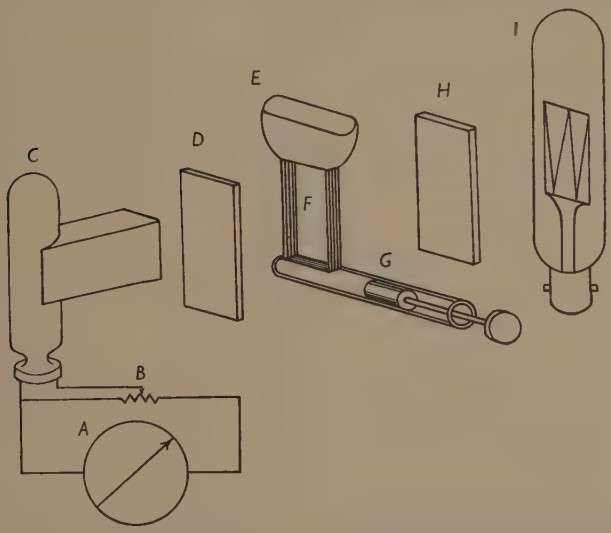

Fig. 1. Apparatus for recording flow orientation of spermatozoa by optical method. Description in text. *A*, spot galvanometer; *B*, sensitivity control; *C*, photocell; *D*, neutral filter; *E*, reservoir; *F*, sample cell; *G*, syringe; *H*, heat filter; *I*, lamp.

since suspensions of spermatozoa show marked flow-birefringence, such as is observed in suspensions of elongated particles. In the case of spermatozoa, optical anisotropy is most apparent when viewing the suspensions in ordinary light, and the use of polarized light, which may be necessary to demonstrate the phenomenon in the case of particles of submicroscopic size, does not accentuate the effect.

In order to demonstrate this flow orientation, by means of optical anisotropy, and relate it to the movement of the spermatozoa in the medium, the instrument shown diagrammatically in Fig. 1. was made. A glass cell (*F*) was made out of cover-slips (1 × 2½ in.) about 0·1 mm. apart and cemented at the edges so that four

separate and parallel films of liquid could be examined together. This composite cell was connected to a syringe below and to a reservoir above, so that when the plunger of the syringe was worked the semen could be made to flow vertically between the cover-slips from the syringe to the reservoir and vice versa. This cell was interposed between the lamp and photo-electric cell of a 'Spekker' differential absorptiometer. The cell was filled with a freshly collected sample of bull semen, and the plunger worked a few times to get rid of air bubbles. The cell contents were then left a few minutes to come to rest. Since a strong beam of light illuminated the cell, 'wave-motion' could be detected easily by the naked eye, the suspension showing the characteristic mottling, which when closely watched could be seen to change its configuration continuously. The sensitivity of the spot-galvanometer was now increased to give a maximum deflexion and the iris diaphragm between the lamp and the compensating photo-electric cell (not shown in the diagram) opened to bring the galvanometer spot back again to zero. The plunger of the syringe was now worked fairly rapidly, causing the suspension to flow. At once the mottling of the semen disappeared; the suspension became more translucent and a positive deflexion on the galvanometer was recorded. If the plunger was worked rapidly (about 2 cyc./sec.) the galvanometer deflexion remained relatively constant, although at the end of each stroke the velocity of flow would be momentarily zero. On stopping the plunger the semen returned to its normal configuration at rest, viz. the galvanometer spot returned to zero, the suspension became more opaque, and the mottling and wave-motion reappeared.

The same procedure was repeated on the same sample of semen at intervals of 15 min. The only change noticed was that as the semen aged, the time interval (recorded with a stop-watch) between stopping the plunger and the return of the galvanometer spot to zero became progressively longer. This interval we have termed the 'relaxation time'. Relaxation time in the strict sense used in physics is the mean time required for orientated particles to regain a certain degree of random distribution of orientation when the orientating force is relaxed. Relaxation or disorientation of small inert particles is due to thermal agitation or Brownian movement. In the case of sperm suspensions, however, Brownian movement is slight and disorientation is due to the movement of the spermatozoa themselves. 'Relaxation time' as recorded here is not the mean time taken by individual spermatozoa to become disorientated, but the total time taken for the suspension as a whole to reach the same degree of disorientation as was originally present in the suspension before the orientating force was applied. The gradual increase in relaxation time which occurs as a semen sample ages, follows the gradual cessation of motility of the spermatozoa. Cessation of motility in a semen sample may be due to exhaustion of metabolites originally present in the semen, to accumulation of lactic acid, or to impairment of cell structure, depending upon the conditions prevailing in the suspension. Dead spermatozoa, and those killed by heat, formalin, etc., gave a maximum relaxation time. Flow orientation is not significantly affected by immobilization or death of the cell. There is the same increase in translucency as measured on the galvanometer. When completely relaxed, however, dead semen

shows little or no silky mottling in incident light, and wave-motion is absent when a layer of semen is examined by transmitted light.

Since relaxation time was clearly related to motility, in subsequent experiments relaxation time and motility were measured together on the same sample of semen. The portable impedance bridge devised by Rothschild (1948, 1949*a*) was used. This instrument records impedance changes, when the electrodes are placed in a dense suspension of active spermatozoa. The frequency of impedance change is closely related to wave-motion, and to the visual assessment of motility (Rothschild, 1949*b*).

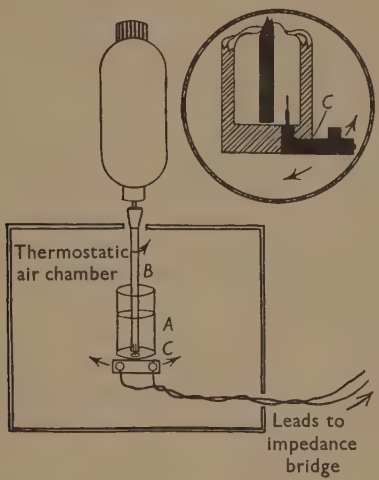

Fig. 2. Apparatus for recording flow orientation of spermatozoa by changes in electrical impedance. Circular inset, details of electrodes in base of cell. Description in text.

Impedance change frequency was measured just prior to the determination of relaxation time by placing the electrodes in the reservoir of the optical cell. Several experiments on different samples of semen were made. It was found that impedance change frequency and relaxation time were closely and inversely related (see results below). It was, however, observed that when the plunger was worked and the semen in the optical cell made to flow, large impedance changes were recorded on the apparatus. This suggested that impedance changes might themselves be due to flow orientation, and it was decided to follow up this possibility.

Trials were made with various types of electrode placed in tubes of different design through which semen could be made to flow. These trials proved unsatisfactory, owing principally to temperature fluctuation and changes in impedance which occurred when taps were opened or the apparatus mechanically disturbed. Eventually the apparatus shown in Fig. 2, which is similar in principle to the Couette viscometer, was tried and gave reasonably consistent results. The cell (*A*) was cylindrical (about 1 in. internal diameter and $2\frac{1}{2}$ in. high) and was of Perspex. Flow orientation was affected by means of a central glass rod (*B*) rotated at a slow

speed by an electric stirrer. Half-way between the glass rod and the cell wall, the electrodes (platinized platinum wires) were mounted on a pivot (*C*) which could be rotated so that the electrodes could be placed either parallel to the flow or at right angles to it. The electrodes were connected by flexible leads to the impedance bridge in the usual way.

When readings were to be taken, the cell was filled with about 6–10 ml. freshly collected bull semen. The semen was stirred for about 15 min., during which time thermostability was attained (temp. *c.* 30° C.). Stirring was then stopped, and after the sample had come to rest the impedance bridge was balanced, and with the sensitivity of the instrument at a maximum, the impedance frequency was measured over a period of 1 min. The sensitivity was then reduced to a value which prior trials had shown would produce on orientating the spermatozoa, a widening of the oscilloscope trace which would lie within the scale set in front of the oscilloscope tube. At this sensitivity the impedance changes due to wave-motion were barely detectable. The stirrer was then switched on. Immediately the oscilloscope trace opened and the width was noted on the scale. On a time signal being given the stirrer was switched off and the interval between the time signal and the return of the oscilloscope trace to balance was recorded with a stop-watch. The relaxation time thus recorded includes the lag due to the momentum of the stirrer and the flowing suspension. As, however, with active spermatozoa, relaxation times of 1–2 sec. were recorded, the error due to lag is negligible. The extent to which stirring gave rise to unbalance of the impedance bridge was recorded by calibrating the width of opening of the oscilloscope trace against similar changes produced by altering the finer resistances, which were graduated in ohms, or fractions of an ohm. A unit of unbalance was taken as that which was equivalent to 1 ohm change of resistance. This method of calibration is probably not accurate, and the units of unbalance are given for rough comparison only. Readings of unbalance were taken with the electrodes parallel to the flow of the suspension and at right angles to it. In the first position the unbalance was approximately 1 and in the second position approximately 0·5. These figures did not vary appreciably from sample to sample of bull semen, but significant differences were recorded with other material (see p. 527).

Readings of impedance change frequency, unbalance produced by stirring, and relaxation time were repeated at 30 min. intervals for 3–6 hr., depending upon the survival of motility of the sample.

RESULTS

An experiment with the optical cell is shown in Fig. 3. Relaxation time in seconds is plotted against the age of sample. Relaxation time is very short at the beginning of the experiment when active wave-motion is also visible, and it increases exponentially until at the close of this experiment it is very long and wave-motion has disappeared. Experiments were repeated with this type of apparatus with different samples of semen, and with simultaneous measurements of impedance frequency on the Rothschild apparatus. Although the time of survival of the sample varied,

there was always a close inverse relation between relaxation time and impedance change frequency. An experiment with the Couette viscometer type of cell is shown in Fig. 4. Relaxation time was measured by recording the interval between stopping

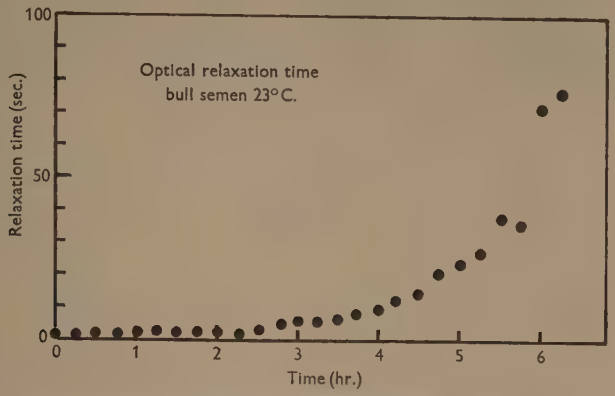

Fig. 3. Relaxation time, as measured by light transmission, plotted as a function of ageing of the sample of semen.

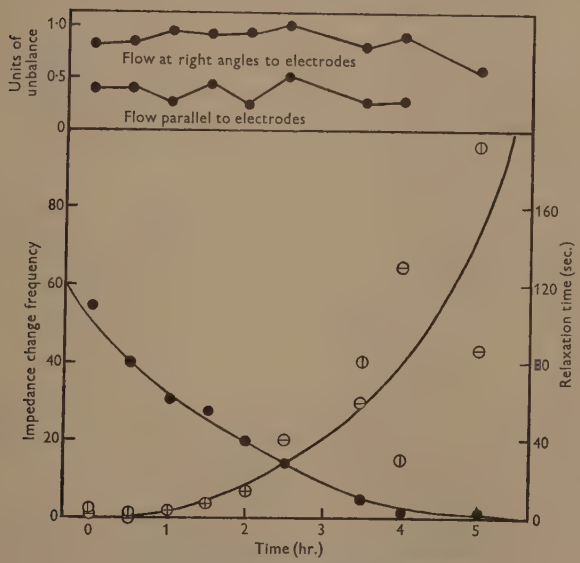

Fig. 4. Impedance change frequency, unbalance, and relaxation time as functions of age of semen sample.

the stirrer and the return of the oscilloscope trace to balance. Relaxation time was measured twice at each determination, once with the electrodes parallel to the flow and once with the electrodes at right angles to the flow. These plots are shown by

circles with horizontal or vertical bars respectively. With the electrodes at right angles to the flow, i.e. placed radially with respect to the cell, the impedance change on orientation was about + 1 unit of unbalance. With the electrodes parallel to the flow, i.e. placed circumferentially with respect to the cell, the impedance change on orientation was about 0·5 unit of unbalance. This is shown at the top of the diagram. No significant difference was noted in the relaxation times recorded when the electrodes were in different positions. Experiments were repeated with this type of

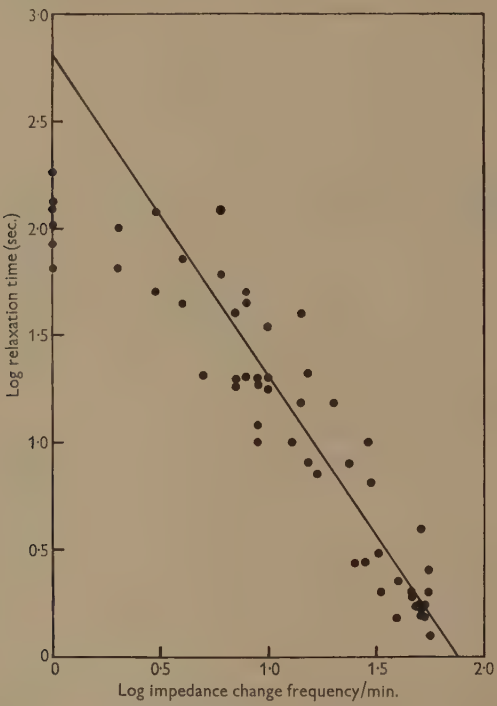

Fig. 5. Double logarithmic plotting of relaxation time against impedance change frequency.

apparatus with different samples of semen. As with the optical measurements, the time of survival of the sample varied, but there was always a close inverse relation between relaxation time and impedance change frequency.

As relaxation time is a characteristic of the semen sample, and independent of the way in which it is measured, all data from both sets of experiments have been plotted on the same log-log scale and shown in Fig. 5. It is apparent that both might be exponential functions of ageing, impedance change frequency decreasing and relaxation time increasing, and that the relationship between the two functions is close and probably linear. The only points which obviously fall off the line are the measurements of relaxation time at very low impedance change frequency. This,

however, might be expected. When relaxation time exceeds several minutes it becomes increasingly difficult to determine the exact time when relaxation is complete, since the galvanometer spot and the oscilloscope trace approach zero asymptotically. Also, the null readings of the instruments may change if a long interval intervenes. The relaxation times at low impedance change frequency shown on the graph have therefore probably been underestimated. By extrapolation of the main body of data, relaxation time at zero impedance change frequency would be of the order of 10 min.

In order to test the validity of the conclusions drawn from the above experiments, namely, that flow orientation of the spermatozoa causes impedance changes, similar to, if not the same as, those produced by wave-motion, and that relaxation time is a function of the motility of individual cells, similar measurements were made with other suspensions in the rotation cell. Cell-free seminal plasma, phosphate buffer and saline solutions gave no significant changes in impedance on stirring and consequently no relaxation. A concentrated suspension of tobacco mosaic virus gave no spontaneous fluctuations in impedance, such as that associated with motile spermatozoa. Stirring, however, produced impedance changes of about 1·3 arbitrary units of unbalance. This may be attributed to flow orientation of the elongated virus particles and electrical anisotropy of the material. Relaxation time was about 129 sec., which can be attributed to thermal agitation, and this value remained constant (tested over a period of 4 hr.) as one would expect with a non-motile organism in which relaxation is not due to motility which shows progressive decay. A concentrated washed culture of *Glaucoma piriformis*, which is an elongated ciliated motile organism, gave all the reactions encountered with spermatozoa. Spontaneous impedance changes of frequency 71/min. of small amplitude were detected at the start, when the organism was very active, and fell to 21/min. after 1 hr. 20 min., when the organism was considerably less active. Stirring produced large impedance changes of about 6 units of unbalance. Relaxation time increased from 80 to 420 sec. from start to finish of the experiment (1 hr. 20 min.).

These experiments with other materials, therefore, substantially confirm the conclusions drawn from the experiments with semen.

DISCUSSION

The results obtained by the optical method demonstrate that flow orientation induced mechanically in concentrated sperm suspensions gives rise to an increase in the amount of light transmitted through the suspension at right angles to the direction of flow of the orientated spermatozoa. This increase might be due either to decrease in the amount of light absorbed, but is more likely to be due to a decrease in the amount of light scattered away from the path of the incident light beam. It follows, therefore, that the light and dark waves which are observed when viewing wave-motion either in transmitted or incident light are not necessarily due to differences in sperm concentration, caused by spontaneous aggregation and disaggregation as suggested by Rothschild (1948). It is possible, however, that changes in concentration do result from orientation and wave-motion (see below). Similarly,

the large impedance changes observed in the rotation cell cannot be due to local changes in sperm concentration between the electrodes but to electrical anisotropy of the orientated material (Rothschild, 1948).

On the basis of the results obtained it is possible to present an hypothesis to explain how wave-motion originates and is maintained in active sperm suspensions, as, for example, in freshly ejaculated semen. Spermatozoa are immotile in the epididymis and vas deferens (Simeone, 1933; Gunn, 1936; Lardy, Hansen & Phillips, 1945), but since immotile spermatozoa are orientated by flow it can be

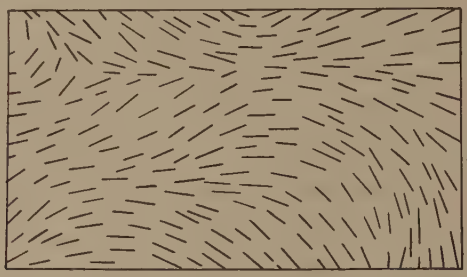

Fig. 6. Short-range order of short-rod molecules (from Kratky, 1934).

assumed that on being impelled through the male tract and mixed with the secretions of the accessory glands, the cells will be flow-orientated before they acquire motility. This orientation will be relatively stable, since we have shown that the relaxation time for immotile spermatozoa is greater than that for motile spermatozoa, and, in the ejaculate, is about 10 min. However, even if the inactive sperm suspension were to become completely relaxed it does not follow that it would become completely disorientated. Kratky (1934) has shown that rod-shaped molecules in suspension are not completely disorientated at rest, but assume a 'short-range order' as illustrated in Fig. 6. A very similar short-range order can be detected in immotile dense sperm suspensions.

Fig. 6 may be taken to represent roughly the configuration of a sperm suspension incompletely disorientated before motility starts. If now the spermatozoa begin to move, it is obvious that those with heads pointing in one direction will tend to separate from those with heads pointing in a different direction, and that streams of spermatozoa will begin to flow in various directions at random. These streams will themselves induce flow orientation. In observing wave-motion it can be seen that streams capable of carrying inert particles, bubbles, etc., do actually form.

In the sperm suspension, eventually, an equilibrium must be established such that the forces tending towards orientation, namely, 'short-range order' and flow, are balanced by the disorientation produced by the random directions taken by the streams, but in small regions of the suspension, e.g. between slide and cover-slip or between electrodes, statistical variation in the direction, and velocity of the streams set up by sperm movement, will create local disturbances of equilibrium,

and turbulence or wave-motion will result. When flow orientation of considerable magnitude is imposed on the system as in the optical cell or the rotation cell, the equilibrium is altered in the direction of complete flow orientation throughout the suspension. When mechanically induced flow ceases, the original unstable equilibrium between orientation (short-range order or sperm flow) and disorientation (random stream flow) is restored and wave-motion returns. The rate of return to unstable equilibrium (relaxation time) is a function of the motility of the spermatozoa.

In discussing, above, the origin of the light and dark waves in wave-motion, and attributing them to optical anisotropy rather than to differences in concentration, it was suggested that changes in concentration might result from orientation. This statement requires explanation. The spermatozoon is not symmetrical about its long axis. In the bull, ram, rabbit and other commonly studied mammalian species, the head is flattened. The tail is morphologically symmetrical or nearly so, and it has been often assumed that the waves of contraction pass spirally down from head to tail. If, however, spermatozoa of the bull are examined in a buffered fructose medium containing 4–7% of polyvinylpyrrolidone, a substance which is very viscous in solution but translucent and apparently inert, the movement of the spermatozoa can be slowed down very considerably without markedly affecting their survival. It is then seen that the waves of contraction are in the same plane as that of the head. This asymmetry of the spermatozoon head and movement of the tail may considerably alter the extent to which orientated spermatozoa can approach one another without mutual interference of movement. Hence orientated spermatozoa may become more densely packed than those moving at random, and the density may be different in different directions relative to the plane of the head.

With regard to rheotaxis it is obvious that flow orientation will account for some features of this phenomenon, namely, the alinement of the spermatozoa in the direction of flow of the medium. Some explanation is required to account for the apparent unidirectional orientation against the flow of the current, and for the finding of Yamane & Ito that the rate of movement of the spermatozoa is a function of the rate of flow. It is possible that these findings arise from errors of observation. If a current is flowing across the field of the microscope at a velocity equal to that of the spermatozoa, only those spermatozoa moving against the flow of the current will remain visible within the field of observation. Spermatozoa moving with the current will be rapidly swept away. The greater the flow of the current the more selective will the current be, and hence the velocity of the spermatozoa observed will appear to increase with the rate of flow. It is, however, also possible that if spermatozoa are orientated and moving in the same direction, parallel to one another, mutual interference is eliminated and their velocity in this direction will be increased for the same expenditure of energy.

In a sperm population, in which the velocities of movement are variable, orientated movement must result in some segregation according to speed of movement, spermatozoa of like velocity keeping pace with one another. This is consistent with the observation of Blom (1946), that dead cells seem to accumulate in strands

throughout suspensions showing wave-motion, but we cannot agree with this author's interpretation that wave-motion is actually due to the presence of dead spermatozoa and the formation of barriers against which the live cells exert pressure. In our view segregation of dead cells is a consequence of wave-motion and not a primary cause.

Flow orientation and rheotaxis may have some bearing on the mechanism of ascent of the spermatozoa in the female tract and on fertilization, but this is at present hypothetical.

SUMMARY

Flow orientation of spermatozoa in dense suspensions has been demonstrated by optical and electrical methods.

Relaxation time, i.e. the time required for a suspension of elongated cells, which have been orientated by flow, to regain a certain degree of random distribution when flow ceases, has been shown to be closely and inversely related to motility, as measured by impedance change frequency in the suspension.

Flow orientation may provide a physical explanation of 'wave-motion' and 'rheotaxis' of spermatozoa.

Wave-motion occurs in a suspension of elongate cells which possess progressive motility in the direction of the long axis of the cell. Orientation of the cells may be brought about by 'short-range order' or flow. Disorientation may be brought about by streams of orientated cells moving in random directions. In the suspension as a whole an equilibrium will be reached such that the forces tending towards orientation (short-range order or flow) are balanced by forces tending towards disorientation (streaming). In small regions, however, statistical variation in the direction and velocity of the streams set up by sperm movements will produce local disturbance of the equilibrium and turbulence or wave-motion will result.

The author is indebted to Mr A. V. Guntrip for his skill in making the apparatus and for help with the experiments. Mr R. C. Campbell advised on the statistical examination of the data and their graphical representation. Lord Rothschild gave valuable criticism of the paper, and advice on the use of the impedance bridge. Dr Markham, Molteno Institute, kindly provided the sample of tobacco mosaic virus, Mr Ryley, Molteno Institute, the sample of *Glaucoma piriformis* and Messrs May and Baker the sample of polyvinylpyrrolidone.

REFERENCES

ADOLPHI, H. (1905). Die Spermatozoen der Säugetiere schwimmen gegen den Strom. *Anat. Anz.* **26**, 549.

BLOM, E. (1946). A comparing-chamber for microscopic examination of undiluted bull semen. *Vet. J.* **102**, 252.

COLE, F. J. (1930). *Early Theories of Sexual Generation.* Oxford.

GUNN, R. M. C. (1936). Fertility in sheep, artificial production of seminal ejaculation and the characters of the spermatozoa contained therein. *Bull. Coun. Sci. Industr. Res., Aust.*, no. 94.

HENSEN, V. (1876). See Adolphi (1905).

KRAFT, H. (1890). Zur Physiologie des Flimmerepithels bei Wirbelthieren. *Pflüg. Arch. ges. Physiol.* **47**, 196.

KRATKY, O. (1934). Zum Deformationsmechanismus der Faserstoffe. II. Die Ordnung der Mizellen von Filmen in kleinsten Bereichen. *Kolloidzschr.* **68**, 347.

LAING, J. A. (1945). Observations on the characteristics of the semen in relation to fertility in the bull. *J. Agric. Sci.* **35**, 1.

LARDY, H. H., HANSEN, R. G. & PHILLIPS, P. H. (1945). The metabolism of bovine epididymal spermatozoa. *Arch. Biochem.* **6**, 41.

LOTT, G. (1872). See Adolphi (1905).

ROTH, A. (1904). Zur Kenntniss der Bewegung der Spermien. *Arch. Anat. Physiol., Lpz.*, Abt. 366.

ROTHSCHILD, LORD (1948). The activity of ram spermatozoa. *J. Exp. Biol.* **25**, 219.

ROTHSCHILD, LORD (1949*a*). Measurement of sperm activity before artificial insemination. *Nature, Lond.*, **163**, 358.

ROTHSCHILD, LORD (1949*b*). Electrical measurement of bull sperm activity. Comparison with visual assessment. *J. Agric. Sci.* **40**, 82.

SIMEONE, F. A. (1933). A neuromuscular mechanism in the ductus epididymidis and its impairment by sympathetic denervation. *Amer. J. Physiol.* **103**, 582.

YAMANE, J. & ITO, T. (1932). Über die Geschwindigkeit der Pferdespermatozoen in strömenden und nicht-strömenden Flüssigkeiten. *Cytologia, Tokyo*, **3**, 188.

THE RANDOM ELEMENT IN BIRD 'NAVIGATION'

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(*Received* 6 *February* 1952)

(With Seventeen Text-figures)

1. INTRODUCTION

The results of experiments on the homing of birds cannot be interpreted simply; those so far available do not indicate unambiguously the existence of real navigational power, and it remains an open question whether or not they can be explained on the basis of search for known landmarks.* This search could be systematic; both radial scatter (Claparède, 1903) and spiral search (Hodge, 1894) have been suggested (Griffin, 1944): on the other hand, the possibility of random search seems to have been discarded by most workers in this field. It does seem at first sight most unlikely that a completely random wandering should be able to explain the experimental results, but this possibility must be investigated more closely for it constitutes the crudest possible method by which home may be reached, and it must be completely excluded if more elaborate speculations are to be justified. It is the purpose of this paper to present such an investigation; it will be shown that random search can indeed explain the observed phenomena associated with bird movements on a large scale.†

2. THE EXPERIMENTS TO BE DISCUSSED

The experiments with which this investigation will concern itself were performed chiefly by Griffin and Rüppell; they have been conveniently summarized by Griffin (1944) and by Matthews (1948). The results are commonly displayed in two graphs: one gives the percentage of the birds released which reach home (the percentage return or return probability) as a function of the distance of the release point from home, and the other relates the average speed of return to this distance. The first graph shows the features which one would expect of it on almost any view of the mechanism of homing, namely, a falling off of percentage return with increasing distance. The rate of fall-off varies considerably from species to species; herring gulls may return in numbers as great as 75% from 1000 miles away, while starlings suffer almost total loss at this distance. The second graph shows more unexpected features: the average speed of return shows no characteristic variation with distance of release; sometimes it remains almost constant, sometimes it increases, and rarely, if ever, does it fall as the distance is increased. The speed itself, however, is low;

* It must be remarked immediately that this investigation concerns itself solely with the homing of wild, untrained birds; the domestic pigeon or other birds which receive a special training are not considered.

† The problem of random search is explicitly discussed in this paper in terms of bird movements, but the results may be applied to the 'homing' of any animate or inanimate object by a suitable choice of the parameters involved.

it commonly amounts to as many miles per day as the bird is capable of flying in an hour, or, at most, two or three times this number. Now this trend of speed with distance is very striking; it has led some investigators to suppose either that the birds scatter radially, so that those which reach home do so with a speed which does not depend on the distance, or that real navigation is involved. It is argued that spiral search would give a speed falling off rapidly with distance, because the length of flight path along the spiral increases more rapidly in proportion than does the distance of home; this is true. It is also argued that random search would also give a speed which falls rapidly with distance; this would also be true if every bird reached home, but it is not true when we take account of the fact that only a fraction of the birds reaches home and that it is with the average speed of that fraction that we must concern ourselves.

It will be seen from what follows that the hypothesis of random search explains the features of both graphs, percentage return and speed, both qualitatively and quantitatively, under assumptions about the behaviour of the birds whose plausibility forms the only question at issue.

Another noteworthy feature of the experimental results is that when birds are released at considerable distances none at all returns for a time which is considerably greater than that appropriate to continuous flight in a straight line. This is put forward as an argument against the hypothesis of random search; it is asserted that a few birds would chance to fly almost straight home and so turn up in a time little longer than that required for flight in a straight line. Such chance flights are possible, but we shall show that the probability that they should happen is quite minute and that, on the contrary, random search would lead to results of just the character observed—a considerable delay and then a quite sudden setting-in of returns which reach a maximum rate and then decline. One may not, then, use this observed waiting period as evidence in favour of a real navigational ability with a flying time of only an hour or two per day.

3. THE ASSUMPTIONS UNDERLYING THE DISCUSSION OF RANDOM SEARCH

The assumptions on which this investigation are based will now be stated:

(i) *The birds search for home independently.* If they do not it does not affect the calculation so long as they do not fly in a body or in a very few large groups. This assumption is made plausible by the experimental results; birds usually return home singly even when released as a group.

(ii) *The search is completely random.* This means that a bird does not recognize territory through which it has wandered earlier in the homing flight should it chance upon it again, but searches it as diligently as before. This assumption is not very plausible for a bird may well profit by its experience, but the making of the assumption ensures that the results of the calculation be pessimistic; random search coupled with memory would give greater percentage returns and higher speeds than those computed here. The assumption also means that the bird will search completely inappropriate territory as thoroughly as that in which its home may

conceivably lie; it will, for instance, examine flat open country when it knows its home to be in wooded hills. This causes the calculation to be pessimistic in the same sense as before. In the following discussion we shall sometimes see what happens when we delimit territory within which the search may be made by a barrier through which the birds do not penetrate but which turns them back into the area of search. This barrier may be so great and unbroken an extent of territory of a sort that cannot possibly contain home that the bird recognizes that further search in it is profitless. This assumption, when made, weakens the hypothesis of random search because the search is only random within a prescribed boundary which may, for example, be the edges of a continent; all unsuitable territory within the boundary is, however, still searched.

(iii) *The mode of random search employed is to fly in a straight line for a certain distance λ, then turn, all angles of turn being equally probable, then fly another stretch in a straight line, turn again and so on.** The bird will fly n such stretches in unit time. It is not assumed that all the stretches are of equal length; the results depend not so much on λ, the length of a single stretch, as on the mean square of these lengths, which we shall call Λ^2. The quantity which determines the success of the search is a combination of Λ and n, namely, $\kappa = \frac{1}{4}n\Lambda^2$, which we shall call the diffusivity. If the distribution of λ is not excessively wide the mean and the root-mean-square lengths of a straight flight are roughly the same and $\kappa \sim \frac{1}{4}vL$, where v is the speed of the bird's flight and L is the mean length of a straight flight before turning. A specific assumption about the distribution of flight lengths would be needed to relate κ and v accurately, but this is not necessary in the present investigation, where nothing is aimed at but to demonstrate the plausibility of a rather general thesis. Generally speaking the greater the value of κ, the greater will be the bird's success at homing. In guessing values for κ we may be guided by two principles: the first is that the speed of flight is known to lie somewhere within fairly narrow limits for each species; we are not free to assume a flight speed of 60 m.p.h. for a starling, though this figure may be possible for a swallow; the second principle is that L should not be enormously greater than the distance of the visible horizon.† A bird flying at a height of 400 ft. has a visible horizon of more than 20 miles in any direction over smooth country; it would therefore be scarcely plausible if, to gain agreement between the theory and the facts, we had to assume a value of L as great as 200 miles. So an upper limit for κ would seem to lie around 3000 miles squared per hour, and in general we should like to be able to explain the results with a considerably smaller value of the diffusivity. Throughout this work distance is measured in miles and time in days, so κ will be measured from now on in miles squared per day with an upper limit of about 72,000.

(iv) *The search effectively lasts only for a finite time.* If the time of search were unlimited all the birds would come home sooner or later. In practice they are not

* It is not suggested that, in practice, these flights are actually straight, they merely do not contain a major change of flight direction such as is supposed to terminate them.

† A limit of another type is laid on L by the mathematical methods adopted in this work; when home is a small region enclosed by a continent L must not be many times as great as the linear dimensions of that region.

observed to do so. Either they die or they get discouraged or so great a time elapses that the incentive for return disappears or the experimenter gives up observation at the return point. Now the law governing the way in which birds drop out of the search cannot be specified; probably a few drop out very quickly and others at greater intervals. We shall assume in this discussion that all the birds search for a time not greater than t_0; those which have not reached home by this time all give up simultaneously and we never hear of them again. This assumption is artificial, and would only be correct if the sole factor operating were the giving up of observation. But the details of the way in which the birds fall out of the search do not affect the conclusions very profoundly. Another simple assumption would be that the birds fall off exponentially, that is to say, that the probability of any bird giving up at any time is independent of the time for which it has already searched; this assumption yields results very similar to those about to be presented. So long as the birds fall off in some way involving a characteristic time the results do not depend much on the details.

A complication enters our discussion of a suitable value for t_0; t_0, as we shall always use it in this paper, is the limiting time which the bird spends actually in the air in active random search for home; this will be less than the time between release and giving up by a factor f. $1/f$ is just the fraction of a calendar day which is spent in flight. f cannot be estimated from our present knowledge of bird behaviour, but it is unlikely to be less than 2 and there seems no reason why it should be greater than 4, which corresponds to 6 hr. of flight per day. If, then, the observations are continued for 60 days t_0 may plausibly be placed between 15 and 30 days. f must also enter our discussion of the speed of return; in the calculations to be presented the speed of return is determined by the time for which the bird actually flew and must be divided by f in order to find the speed as recorded in the actual experiment. Whenever numerical results are presented and demand a definite figure, we shall assume $f=3$—a flying day of 8 hr.

4. A REMARK ON THE AVERAGE SPEED OF HOMING

The experimental results on the average speed of homing are frequently arrived at by taking the time of return of each bird to the nearest day. In order to achieve greater flexibility—independence of f—we have not followed this method in these calculations, but have given the true average speed, using the exact time of return; this increases the average speed for small distances of release—our curves show a higher speed from small distances than they would were the usual method of presentation to be adopted. This effect will be the greater the smaller the value of f and should be borne in mind in comparing these calculations with experiment.

5. DIFFERENCES IN THE HOMING OF LAND BIRDS AND SEA BIRDS

We shall assume that land birds wander aimlessly in the manner described above; they are not permitted to use any geographical or ecological aids but continue their random search until they encounter known territory. We shall assume that sea birds, however, are able to recognize a coast-line as a possible site for their home; a

sea bird released on a coast is assumed to stick to it fairly closely and wander up and down it until known territory is encountered. The bird may be imagined confined to a strip adjacent to the coast, but of width comparable with L, in which case the appropriate κ is that given in §3(iii); it may, alternatively, be imagined to cleave to the coast itself, in which case the appropriate κ is $\frac{1}{2}n\Lambda^2$ rather than $\frac{1}{4}n\Lambda^2$. The distinction is not important. The former method allows the search to be somewhat more flexible and would permit, for instance, the changing of coasts should another present itself within a distance not very much greater than L of that along which search is being presently conducted; the latter method is rather quicker as κ is twice as big for the same flight constants, n and Λ, but it confines the bird to a coast once chosen; in practice birds may well use a combination of the two methods. If a sea bird is released inland its search is imagined to be, first, a random one for a coast, and then a random one along the coast until known territory is reached.

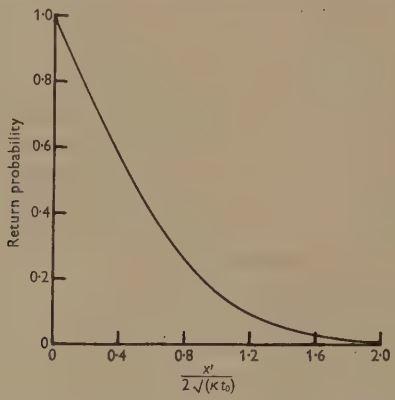

Fig. 1. Return probability as a function of $\frac{x'}{2\sqrt{(\kappa t_0)}}$ for coastwise homing.

6. THE HOMING OF SEA BIRDS ALONG A COAST

The first result to be presented refers to sea birds which are released at a distance x' from home on a semi-infinite coast-line.* The birds are free to wander as far away from home as chance dictates. The results for all values of κ, x' and t_0 may be summarized in a single graph. Fig. 1 shows the percentage return plotted as a function of $\frac{x'}{2\sqrt{(\kappa t_0)}}$ (the analytical form is given in the Appendix, §14). To facilitate comparison with experiment Fig. 2 shows the percentage return plotted as a function of x' in miles for several values of κt_0 in miles squared. We have noted in §3(iii) that

* This assumes that the whole coast is unknown. If a certain length of coast about home is presumed known then x' refers more properly to the distance of the point of release from the nearest point of the known stretch. The results of this section on the average speed of homing then refer to the speed of return to the known stretch rather than to home itself, and a small correction to the observed speed of homing may be necessary on account of the flight from the beginning of known territory to home before comparison with the results of the theory based on random search. An example of such a correction is given in § 10.

the highest plausible value for κ is about 72,000 miles squared per day for random homing of the first type discussed in §5; this probably remains a good upper limit even when one considers the doubling of κ for the strict coastal homing of the second type discussed in §5, as it is unlikely that sea birds would approach the 60 m.p.h. used as the upper limit in estimating κ in §3(iii). With a maximum t_0 of about 20 days the maximum likely κt_0 becomes about 1,440,000 miles squared. This corresponds to a return probability from $x' = 1000$ miles of 55%. Fig. 2 agrees in form with the experimental results and predicts a percentage return which ranges

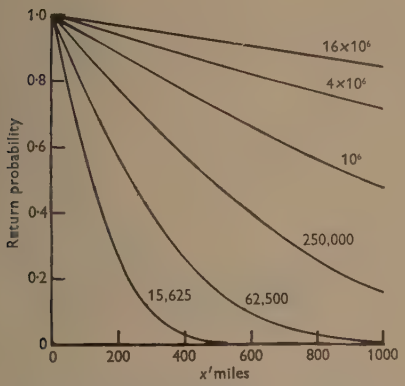

Fig. 2. Return probability as a function of distance of displacement (x') in miles for coastwise homing. Values of κt_0 in miles squared are shown on the curves.

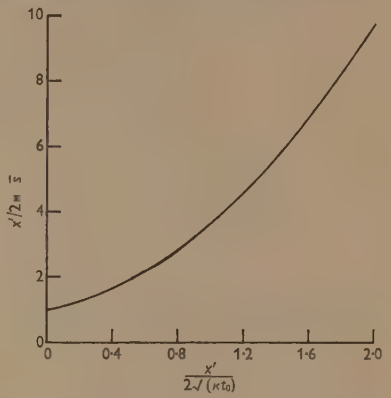

Fig. 3. Average speed of homing ($\bar{s}$) as $\frac{x'}{2\kappa}\bar{s}$ as a function of $\frac{x'}{2\sqrt{(\kappa t_0)}}$ for coastwise homing.

up to the right order for the best performers. We shall soon see that Fig. 2 is pessimistic, and that a higher percentage return should in fact be expected, especially from great distances.

When we turn to the predictions of the theory as to the average speed $\bar{s}$ of return, we find that once again the results for all values of κ, x' and t_0 may be summarized on one curve. Fig. 3 shows $x'\bar{s}/2\kappa$ plotted as a function of $\frac{x'}{2\sqrt{(\kappa t_0)}}$ (the analytical form is given in the Appendix, §14); ease of comparison with experiment is facilitated by Fig. 4, where $\bar{s}/2\kappa$ is plotted against x' for several values of κt_0. $\bar{s}$ is in miles per day. It is seen that the average speed of return does not fall off rapidly with increasing distance in all cases, but that, on the contrary, it may actually rise with distance. When it is borne in mind that the method of averaging adopted here appears to increase the speed for small distances of release (see §4), it will be seen that the curves of Fig. 4 resemble in form those found in practice;* the absolute value of the homing speed is also of right order. Notice also that, whereas the probability of reaching home at all is determined by κt_0, the speed of homing is proportional to κ for a given κt_0 and that $\bar{s}/2\kappa$ does not change very

* See also the next footnote.

rapidly with κt_0; this implies that those birds which home in the largest numbers should also be expected to home with the greatest speed, and this is in accord, in the main, with the facts, though wide variations in t_0 and f from species to species could alter the situation.

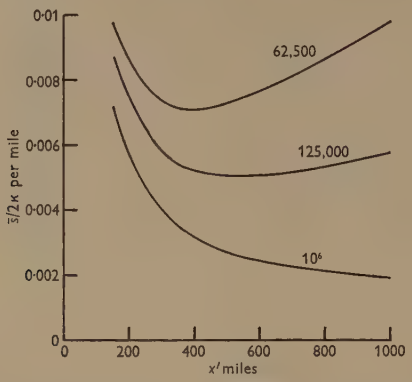

Fig. 4. Average speed of homing as $\bar{s}/2\kappa$ (per mile) as a function of distance of displacement (x') in miles for coastwise homing. Values of κt_0 in miles squared are shown on the curves.

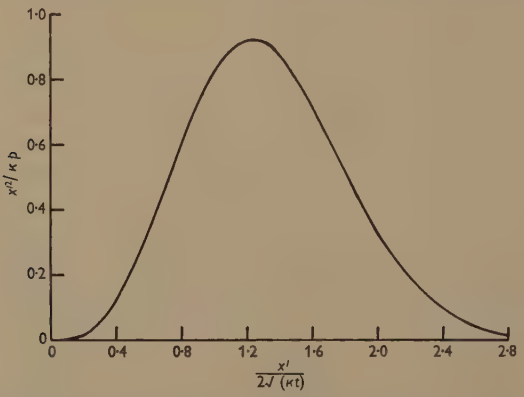

Fig. 5. Probability of return per unit time (p) as $\frac{x'^2}{\kappa} p$ as a function of $\frac{x'}{2\sqrt{(\kappa t)}}$ for coastwise homing.

Another prediction of the theory which it is interesting to examine is the rate of return as a function of time. Fig. 5 shows the probability p of return per unit time plotted as $\frac{x'^2}{\kappa} p$ against $\frac{x'}{2\sqrt{(\kappa t)}}$ (the analytical form is given in the Appendix, § 14). In Fig. 6*a*, p/κ is plotted against κt for several values of x'. (This probability refers

to any single bird of course.) Fig. 6*b* shows the variation of the probability with time in more detail for smaller values of κt. It is seen from Fig. 6*b* that, for the larger values of x' in particular, there is a considerable period of waiting during which the probability of a bird's return is very small indeed. Consider this period of waiting to be terminated by the probability of a bird's arrival having reached 1% and it to be of duration t_w; the time required by the bird to fly straight home is t_f. We are now interested in the ratio $R = t_w/t_f$, the ratio between the observed waiting

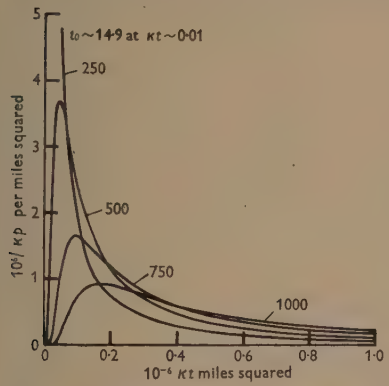

Fig. 6*a*. Probability of return per unit time as $\frac{10^6}{\kappa}p$ (per miles squared) as a function of $10^{-6}\kappa t$ in miles squared for coastwise homing. Values of x' in miles are shown on the curves.

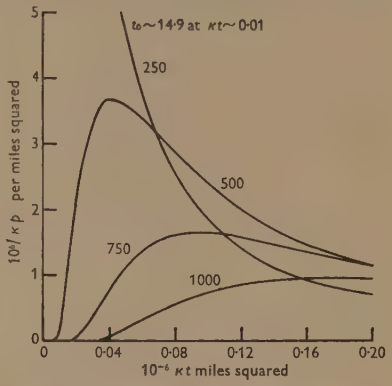

Fig. 6*b*. Enlarged view of the beginning of Fig. 6*a*.

period and the time for direct flight. We have, using the form $\kappa = \frac{1}{4}vL$, $R = 0{\cdot}29x'/L$ provided that x' is considerably greater than L. Thus with $x'/L = 10$, for example, we must wait nearly three times as long before even one bird in a hundred is home as we would think at first sight.* The absence of any returns for a time after release considerably greater than t_f may not therefore be adduced as evidence against random search and in favour of a small number of flying hours per day.

So far we have assumed a rigid division of territory into the completely unknown, in which random search characterized by a diffusivity κ goes on, and the completely known which leads immediately to home. We should, before leaving this question of homing along a line, consider what would be the result of interposing between the completely strange and completely known territories a stretch of

* It should be remarked that the mathematical methods used in the treatment of these problems are approximate (see the Appendix, § 13) and that the approximation is the more accurate the greater the value of x'/L; the sense of the approximation is to increase the average speed for small values of x' by exaggerating the probability of return after short times. Another effect is to make R as quoted here rather smaller than a rigorous treatment would reveal. No sense is to be attached to Figs. 5 and 6 for values of t less than x'/v; a better curve would be given by setting the probability of return equal to zero below $t = x'/v$, taking a curve which starts from the abscissa at this point and fitting it to the given curve at a value of t two or three times as great as this.

territory with which the bird felt some familiarity but which was not well enough known to lead it straight home. We may suppose that the bird would search this familiar territory, which it associated with its home, rather more minutely than the quite unfamiliar territory from which it had come. This is equivalent to a reduction of L, the average length of a 'straight' flight—a reduction of Λ^2 and of κ. (v is supposed to remain constant.) Let us suppose now that this stretch of familiar territory is of length l, and that in it the random search is characterized by a diffusivity ξ times smaller than that in the unknown territory. If $\eta = \xi^{\frac{1}{2}} - 1$, the

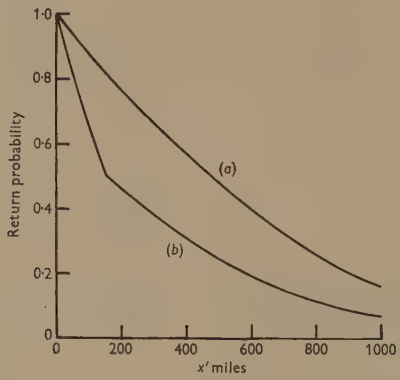

Fig. 7. Effect on the return probability of coastwise homing of interposing familiar territory between known and unknown territories: (*a*) normal homing with $\kappa t_0 = 250{,}000$ miles squared, (*b*) homing with $l = 150$ miles, $\xi = 10$, $\kappa t_0 = 250{,}000$ miles squared.

probability of return from a distance x' from home (the completely known territory) is the same as that for release at a distance $x' + \eta l$ from home in territory characterized uniformly by a diffusivity κ such as we have considered until now. This is for $x' > l$: if $x' < l$ the effective release distance is $\xi^{\frac{1}{2}} x'$; so Fig. 1 may still be used to determine the percentage return (κ is always the diffusivity appropriate to completely unknown territory). The percentage return will obviously drop rather more quickly with increasing distance at first than in the simple case. As an example Fig. 7 shows the variation with distance of the return for the values $\xi = 10$, $l = 150$ miles, $\kappa t_0 = 250{,}000$ miles squared; in the same figure is shown the curve for normal homing taken from Fig. 2 for the same value of κt_0.

The effect of 'familiar' territory upon the speed may be considered; the average speed of return is given by finding the average speed for release at a distance $x' + \eta l$ in the simple case from Figs. 3 or 4 and multiplying it by $x'/(x' + \eta l)$ (for release in unknown territory). Fig. 8 gives the value of $\bar{s}/2\kappa$ as a function of x' for the same values of κt_0 as in Fig. 4, using the same values of ξ and l as in Fig. 7. Comparison of Figs. 4 and 8 shows that the interposition of familiar but not known territory reduces the speed of return for small values of x' and makes the speed either increase uniformly with the distance of release or remain almost independent of it right up to the largest values of κt_0 which we have deemed to be interesting.

It seems quite probable that some such behaviour as that envisaged here should in fact obtain; at all events, it should no longer occasion surprise that the average speed of homing varies with distance of release in the way observed.*

Another effect of this modification is on the 'waiting ratio' R which we have defined and discussed earlier in this section; this increases the value quoted above by a factor $(1+\eta l/x')^2$ (for $x' > l$), and the criterion for its consideration is now that $x'+\eta l$ should be considerably greater than L. For example, with $x'=500$, $L=50$ and values of ξ and l used above, R increases from 2·9 to 7·9.

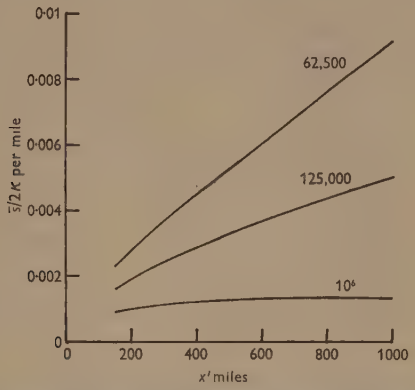

Fig. 8. Average speed of homing ($\bar{s}$) as $\bar{s}/2\kappa$ (per mile) as a function of distance of displacement (x') in miles for coastwise homing under the imposition of familiar territory between known and unknown territories ($l=150$ miles, $\xi=10$). Values of κt_0 in miles squared are shown on the curves.

If we review the conclusions so far drawn about homing along a coast we see a fair measure of accord between the predictions of the theory based on random search and the experimental results. In particular, the dependence of speed on distance is seen to be in reasonable agreement, under plausible assumptions, with what is found in practice. The dependence of percentage return on the various parameters again shows the same qualitative features as the experiments, but the numerical value of the return probability is perhaps not quite so high as could be wished for large distances of release. On looking back to the assumption made as

* A persistent phenomenon, which has impressed many observers, is the abnormally low speeds recorded by birds released in the immediate vicinity of home. A possible explanation, though one which, in view of other simple explanations, we would not press, is suggested by the present considerations. If, as the terrain gets more and more familiar, the bird searches ever more minutely—with an ever-decreasing L—then, by an extension of the above remarks, we should expect the average speed of return to fall, the nearer the point of release to home. Very familiar but not yet known territory would then behave almost as a reflecting barrier and progress through it would be very slow. But it seems to us more likely that the true explanation is based either on complete familiarity with the surroundings—the bird is then 'in no hurry to return'—or on recollections of the trapping and transportation—the bird is then unwilling to return immediately to these unpleasant associations. Alternatively, it may be that the bird, having been trapped from the nest, does not realize, on release, that it is really its spell of duty at the nest and behaves as though it were its turn to seek food; it may not then return to the nest for the usual length of time, which may be days in some species.

to the nature of the coast, however, we see that we have been very ungenerous to the theory in assuming that it was unbounded and of infinite length, allowing the bird to wander indefinitely far from home. In practice it is clear that there will exist at some distance from home some effective barrier such as that discussed in §3(ii); for instance, a bird which lives in a temperate stretch of coast is unlikely to search very far into a region of ice-bound coast nor into tropical seas where the environment is again very different from that in which it knows home

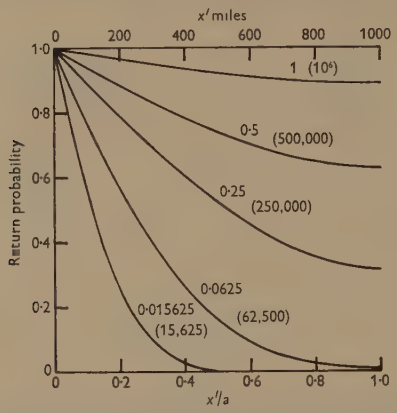

Fig. 9. Return probability for coastwise homing along a bounded coast of length a; lower abscissa scale, as a function of distance of displacement (x') as x'/a; upper abscissa scale, as a function of x' in miles for $a = 1000$ miles. Values of $\kappa t_0/a^2$ are shown on the curves; values of κt_0 in miles squared for $a = 1000$ miles are shown in brackets.

to lie. The effect of imposing such a reflecting barrier at some distance a very remote from home is obviously going to be very small indeed so long as x' rests substantially smaller than a, but may be fairly considerable if x' approaches a. If we impose such a barrier the results can no longer be summarized on a single graph and must be represented as a family of curves. The percentage return is shown in Fig. 9, as a function of x'/a for various values of $\kappa t_0/a^2$ (the analytical form is given in the Appendix, §15). The upper abscissa scale gives x' in miles assuming $a = 1000$ miles, which seems a plausible sort of figure; the numbers in brackets now give κt_0 for this value of a so that a direct comparison with Fig. 2 may be made. It is seen that, for small values of κt_0, Fig. 9 resembles Fig. 2 very closely in all particulars; for large values of κt_0 and large values of x', however, Fig. 9 shows a considerably greater percentage return than Fig. 2. In particular, we see that our limiting value of 1,440,000 for κt_0 now gives a percentage return of over 95% from 1000 miles as compared with the 55% of the unbounded coast.

The probability p of return per unit time is multiplied by a^2/κ and plotted in Fig. 10 as a function of $\kappa t/a^2$ for some values of x'/a (the analytical form is given in the Appendix, §15); the right-hand ordinate scale gives p/κ and the upper abscissa

scale κt for $a = 1000$ miles, so that direct comparison with the numerical values of Figs. 6*a* and 6*b* may be made. The numbers in brackets on the curves now give x' in miles. The curves resemble those of Figs. 6*a* and 6*b* closely in form and also in numerical value for small values of κt and not-too-large values of x'/a. For large values of κt the numerical values of Fig. 10 exceed those of Fig. 6*a* because of the trapping effect of the barrier which prevents birds wandering off to great distances from home. As x' approaches a the numerical values of Fig. 10 rise above those of Figs. 6*a* and 6*b* even for small values of κt, as would be expected, as many birds are now prevented from straying over the barrier even for small values of κt and are turned back in the right direction. The form of the curves, however, still approximates quite closely to that of Figs. 6*a* and 6*b*.

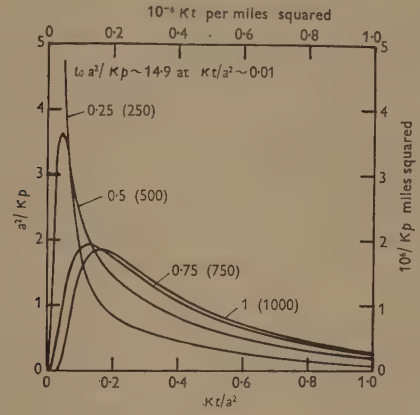

Fig. 10. Probability of return per unit time (p): as $\frac{a^2}{\kappa}p$ as a function of $\kappa t/a^2$ for coastwise homing along a bounded coast of length a (left-hand ordinate and lower abscissa scales); as $\frac{10^6}{\kappa}p$ (per miles squared) as a function of $10^{-6}\,\kappa t$ in miles squared for $a = 1000$ miles (right-hand ordinate and upper abscissa scales). Values of x'/a are shown on the curves: values of x' in miles for $a = 1000$ miles are shown in brackets.

Now the average speed of return is determined only by the form of p and not by its numerical value; this means that the imposing of the barrier has little effect on the dependence of $\bar{s}$ on the various parameters and that Fig. 3 (and Fig. 8) apply at any rate approximately in this problem also, particularly for small values of κt_0. Exact curves may be computed from Fig. 10 or the material presented in the Appendix, §15.

We see that the imposing of the barrier, for which there is good physical justification, leads to numerical values for the probability of successful homing which are in quite reasonable accord with experiment and that the dependence of speed of homing on distance remains satisfactory.

7. THE HOMING OF SEA BIRDS RELEASED INLAND

When sea birds are released inland we suppose that they search at random for a coast and then follow it by either of the methods discussed in the previous section; a complete calculation of the effects to be expected must describe the point of release by two co-ordinates relative to home; the distance of the release point from home and from the coast form such a pair. This complete calculation has not been made; it would be very complex and is not warranted in a general examination of this kind. It is certain that the same general features would emerge as are shown in the results of the previous section; some qualitative remarks should, however, be made.

We imagine the region of search to be bounded, as before, by extensive territory which is qualitatively so different from anything known to the bird that it sees no point in penetrating it; the region for search becomes a rectangle perhaps 1000 miles across in the direction at right angles to the coast, and 2000 miles long parallel to the coast, home being situated at the centre of the 2000 mile coast. If the bird is released considerably nearer to the coast than to home then the appropriate figures of the previous section apply fairly well; x' is again the distance of the release point from home.* Under all other circumstances the effective distance of release is rather greater than the real one, though not by a large factor. The effective value of a will also vary with the position of release, ranging, in our example, from 1000 miles, for release near the coast, to perhaps half as much again for release very far inland. The results of the previous section continue, therefore, to give a good indication of the trends to be expected and also a rough indication of numerical values.

In changing from §6 to the present section, we have introduced a new fiction: §6 deals with homing along a coast, and there was no need, for the purposes of the argument, to assume it to be straight; we are now assuming a model continent with a long straight coast-line and that the birds have no intelligence at all above an ability to recognize that their home lies on a coast and, perhaps, that very exhaustive search in a totally strange type of country is of no use. Since we have not made detailed calculations of this problem, but are rather using the results of the previous one with some reserve, our assumption about the shape of the continent is not very important. The only experiments so far conducted have been made in continents rather deeply indented with large rivers, bays and so on; in these circumstances it is very probable that the bird would pick up some clue rather earlier than it would in our model continent and this would enable the results of our previous calculation to be applied with more confidence.

8. THE HOMING OF SEA BIRDS RELEASED AT SEA

Here we may visualize a natural barrier provided to north and south by temperature changes; but a barrier in longitude is not so easily provided. A barrier in longitude may be found in changes of surface temperature caused by oceanic currents or in

* If the whole coast is presumed to constitute known territory then the appropriate x' will be the distance of the release point from the coast. Care must now be taken in interpreting the speed of homing as given by Fig. 3, since this will be the speed of homing to the coast only. An example of this kind is given in §10.

changes of the composition of the life near the surface of the ocean. It is, however, likely that a bird released at sea would have some idea of the direction in which land lay; this knowledge it would have acquired by flying near its home and noting that the coast lay, let us say, towards the setting sun. We may perhaps assume that the bird would fairly soon find the coast and begin its random search as before. This argument may well be applied to sea birds released far inland and makes a little more plausible our assumption that we are, there also, effectively dealing with coastwise search.

If birds really possess some such faculty, born of experience, for locating the coast fairly directly, then the effective value of x' would be smaller than the distance from release point to home rather than greater, and the effective value of a would be as in §6.

9. THE HOMING OF LAND BIRDS

The birds are supposed to have complete knowledge of all territory within a distance a of their home; should they chance upon this circular area they are able to go home at once. We imagine them to be released at a distance x' from home in unknown territory. As in the case of homing along a coast we have two choices open to us: we may consider the birds to be free to wander as far from home as chance dictates, or we may oblige them to remain within a distance b of home by some natural barrier such as the limit of a continent. The relations between the results obtained under the two assumptions are similar to those developed in §6 in detail for sea birds; until x' becomes comparable with b the results are closely similar, especially for small values of κt; when x' approaches b the returns may exceed those obtained with a boundless continent by a factor of the order two. We present here only those results obtained under the physically more plausible assumption, namely, a continent circumscribed by a circular barrier of radius b centred on home.

The results for the percentage return are rather more difficult to present than for sea birds. If the percentage return is plotted against x'/b for several values of $\kappa t_0/b^2$, then a separate graph is needed for each value of the ratio b/a; as an example Fig. 11 shows such a plot for $b/a = 20$ (the analytical form is given in the Appendix, §16); some possible numerical values are provided by the upper abscissa scale which gives x' in miles for $b = 1000$ miles; the figures in parentheses on the curves give κt_0 for this value of b. A direct comparison with Figs. 2 and 9 may now be made; it is seen that the general trend of behaviour and, indeed, the numerical values are quite similar in all three graphs. It may be supposed that the results in the present problem depend critically on the ratio b/a chosen—on the assumed size of the known territory for a given continent; this, however, is not so. This is, perhaps, a satisfactory feature, for little is known of the size of the known territory. This point is illustrated by Fig. 12 in which the percentage return is plotted, as in Fig. 11, as a function of x'/b, but for the arbitrarily chosen value 1 for $\kappa t_0/b^2$ and for several values of the ratio b/a. It is seen that, for this particular value of $\kappa t_0/b^2$ and for $x'/b = 1$, the percentage return only varies by a factor 1·75 as b/a changes by a factor 8, or, taking $b = 1000$ miles, as the assumed radius of the known territory changes from 100 miles to $12\frac{1}{2}$ miles. The effect may be worked out in detail from

the material given in the Appendix, §16. Our maximum plausible κt_0 now gives a return of over 60% from 1000 miles for $b = 1000$ miles and $b/a = 20$. The probability of return per unit time again resembles Figs. 6*a* and 6*b* both in form and in numerical value. As in the problem of homing along a bounded coast, this means that we may use Figs. 3 and 8 as a fairly good guide to the sort of average speed to be expected. (The problem of surrounding the known territory by a ring of 'familiar' but not known territory has not been treated, but the results would follow closely those for the analogous problem in coastwise homing—§6.)

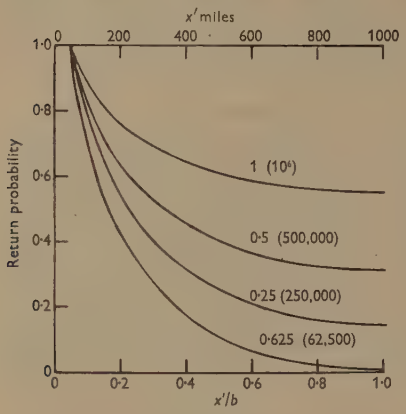

Fig. 11.

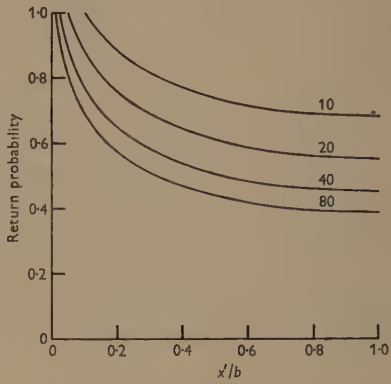

Fig. 12.

Fig. 11. Return probability for homing in a bounded circular continent ($b/a = 20$): lower abscissa scale, as a function of distance of displacement (x') as x'/b; upper abscissa scale, as a function of x' in miles for $b = 1000$ miles. Values of $\kappa t_0/b^2$ are shown on the curves; values of κt_0 in miles squared for $b = 1000$ miles are shown in brackets.

Fig. 12. Return probability for homing in a bounded circular continent as a function of distance of displacement (x') as x'/b. Values of b/a are shown on the curves. ($\kappa t_0/b^2 = 1$).

Something analogous to a known coast for sea birds may sometimes exist for land birds; if a migration route be presumed to be known (or perhaps familiar) territory, then the effective area of home becomes much greater. If the release takes place near the migration route, it is appropriate to use the relations presented in §6, x' being now the distance of the release from the migration route. As we have remarked above, Figs. 2 and 11 are closely similar in numerical value as well as form, so we may conjecture that the appropriate x' to use for homing in a region containing a known migration route would be the distance of the release point to the nearest point of the migration route; there is, however, little evidence for any effect of a known migration route on the homing of land birds.

10. DISCUSSION OF THE RESULTS

We have seen that the principal features of the experimental results from large-scale experiments on the homing of wild birds are explicable on the basis of random search. The only assumptions which must be made concern the speed of flight v

and the value of L, these two making up the diffusivity κ, and the fraction f of a day spent in search. To illustrate the application of the results, let us examine some experimental data and show how they may be fitted to the theory. This fitting, it should be emphasized, does not seek to establish the flight characteristics for the species involved, but only shows that a plausible set of such characteristics may be assembled which gives results in accord with experiment.

It is clear, from the results presented in Figs. 2, 9 and 11, that the fall-off of percentage return with increasing distance of release may always be represented by an appropriate choice of κt_0. It remains to fit the average speed of return; as remarked above, this is not a good constant either experimentally or theoretically. However, a rough figure for the average speed can be derived from the experimental results; where the speed fluctuates irregularly with distance a mean figure has been used, and where there is a definite trend with distance the mean average speed computed over all distances of release has been used. Fig. 4 shows that the average speed at $x' = 500$ miles is a fair measure of the speed predicted by the theory, since the curves are rather flat around this point for the range of κt_0 values required by the results of percentage return versus distance. We use the known value of κt_0 demanded by the percentage return versus distance and $x' = 500$ miles to find from Fig. 3 the value of $\bar{s}/\kappa$, and hence $\bar{s}t_0$. Now the experimental value of the average speed is $\bar{s}/f$ or $\bar{s}t_0/T$, where T is the time in calendar days before the random search is given up in the sense of §3(iv); so the observations of both percentage return and speed may be fitted by postulating a value of κt_0 which leads to a predicted value of T to be compared with that experimentally realized. This comparison is a little difficult to make because T is not well-defined and, as we have pointed out, the assumption of a rigid upper limit to the period of search is only a mathematical convenience, and many other modes of falling out with time would give equivalent results. However, we should at least expect the calculated value of T to agree roughly with the time at which returns are severely falling off; we have therefore compared T with the time after release at which the last successful bird returned and with the time by which 95% of the birds which homed successfully were home.

The following species have been examined: herring gull, starling, Leach's petrel and common tern.* The results are displayed in Table 1. $\bar{s}_{\text{obs.}}$ is the observed average speed and $T_{\text{calc.}}$ the theoretical value of T which this result demands when coupled with the quoted value of κt_0 which satisfies the fall-off of percentage return with distance. $T_{\text{ext.}}$ is the time after release at which the last successful bird returned and T_{95} the time by which 95% of the birds which homed successfully were home. κt_0 is in miles squared, $\bar{s}_{\text{obs.}}$ in miles per calendar day, and the times in calendar days.

The experimental data are from the collations of Griffin (1944) and Matthews (1948) and from the various papers cited. It is seen that the values of T which this interpretation of homing demands are very reasonable and are in good accord with the known return times. It is particularly pleasing that the common tern, which

* Very few experiments are suitable for this type of analysis; the requirements are that fairly large numbers of birds and large distances be involved.

seems here to demand an unusually low value of T, should indeed be found to be associated a low value.

Two more points may be examined. The first is the value of κ demanded by the results; if we assume the reasonable value of 3 for f (and our choice is not restricted by the experimental results), κ ranges from about 8000 to 40,000 miles squared per day; these values fall comfortably within our upper limit of 72,000 miles squared per day established in §3(iii). The second point is the way in which the returns are

Table 1

Species	κt_0	$\bar{s}_{obs.}$	$T_{calc.}$	$T_{ext.}$	T_{95}	References
Herring gull	500,000	80	39	50	21	Griffin (1943)
Starling	100,000	30	38	94	37	Rüppell (1935, 1936, 1937)
Leach's petrel	250,000	40	48	34	22	Griffin (1940)
Common tern	100,000	130	9	16	7	Griffin (1943)

distributed in time; it is obvious from a glance at the detailed experimental results that a great spread in time does exist; this is expected on the basis of random search and is not to be expected on the basis of systematic exploration of radial or spiral type. It is difficult, however, to show that this spread is quantitatively in agreement with the hypothesis of random search. We have displayed, in Fig. 5, the relation between probability of return per unit time p, and the time t, in a manner relating simultaneously to all species (through κ) and all distances of release (x'); but this relation suffers from the disadvantage that it implies all values of the time t and does not include the cut-off after t_0. Nor may the more detailed curves such as Figs. 6*a* and 6*b* be used, because the numbers of birds are small and the change of behaviour with x' is rather rapid. It is most satisfactory to display the experimental results as in Fig. 5, noting that we should expect only the general shape and position of the maximum to agree with Fig. 5, but not the absolute magnitudes because of the finite period of search, which means a return probability of less than that implied in Fig. 5, namely, unity. Fig. 13 shows the results for starlings based on the work of Rüppell (1935, 1936, 1937); the dotted curve is taken from Fig. 5. It is seen that the predicted behaviour accords quite well with experiment; the experimental distribution is of the right shape and position and departs in the way expected from the curve which is appropriate to an infinite search. The cut-off at high values of $\frac{x'}{2\sqrt{(\kappa t)}}$ is due to the birds having a finite speed of flight; they cannot gain home in less than a certain time and so very small values of t $\left(\text{large values of } \frac{x'}{2\sqrt{(\kappa t)}}\right)$ are impossible.

There is, as yet, little evidence on the paths of birds executing homing flights; the most direct comes from the observations of Griffin & Hock (1949) on homing gannets. Nine flight courses were plotted over distances ranging up to 200 miles by following the birds in an aeroplane; they showed no system at all and were what one would have expected had the search been random or very largely so; long fairly straight flights were separated by quite large angles in the way we have assumed

here—§3(iii). This investigation is particularly valuable in two ways: it affords direct evidence for the hypothesis of random search, and it enables an estimate of κ to be made for one species at any rate. From the published data we find $\Lambda^2 \sim 1500$ miles squared, or $L \sim 39$ miles; if we exclude the flights immediately following release—and these are not typical of the whole journey—we find $\Lambda^2 \sim 2300$ miles squared, or $L \sim 48$ miles. These figures, taken together with a flight speed of 35 m.p.h., suggest a value for κ of about 10,000 miles squared per day, if we use $\kappa = \frac{1}{4}vL$; if we use the more appropriate $\kappa = \frac{1}{4}n\Lambda^2$, we find $\kappa \sim 11{,}500$ miles squared

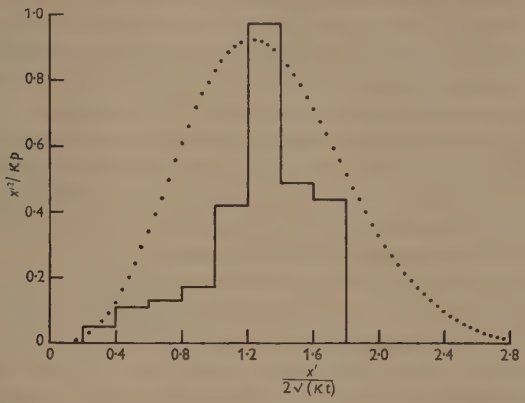

Fig. 13. Probability of return per unit time (p) as $\frac{x'^2 p}{\kappa}$ as a function of $\frac{x'}{2\sqrt{(\kappa t)}}$; dotted curve, from Fig. 5; step function, experimental results with starlings.

per day. It must be remarked that this directly determined value of κ falls within the range inferred immediately above from homing performances alone of some other species and the assumption $f = 3$. Of seventeen gannets released at a distance of 213 miles from home, ten homed successfully—a return of 59%; the longest time taken was 101 hr.—slightly over 4 days; we may then suppose the appropriate value of t_0 for this species to be about $5/f$ days under these conditions. The release point was about 100 miles from presumably known coast. Fig. 2 shows that a 60% return from 100 miles is achieved with a κt_0 value of about 18,000; with $\kappa = 11{,}000$, this gives $t_0 \sim 1{\cdot}6$ days, which is consistent with the observation that the returns were spread over about 5 days and gives an f value of about 3·1. If we turn to the predictions of the theory as regards speed, we find from Fig. 3 $\bar{s} = 360$ miles per day or, with our f value of 3·1, 116 miles per calendar day. This speed is for reaching the coast, which we have presumed to constitute known territory; some time is required to fly along the coast to home, an average value for which distance is about 200 miles and this requires a time of about 6 hr. We may use our value of 3·1 for f to get a rough estimate of the time taken to reach the known coast by subtracting 19 hr. from the observed time taken by each bird to reach home. When this is done we derive an average speed from the experimental results

of 102 miles per day for reaching the coast, to be compared with the theoretical figure of 116 miles per day. These very scanty experimental results are therefore in accord with expectation based on what is here a measured κ for the bird. It would be straining things too far to say more than this, as considerable uncertainties are introduced, not only by the lack of statistically significant data but by the approximation of the problem to one of ideal geometry, spreading the flight uniformly through the 24 hr. and so on.

It is seen from this discussion of the relation between the results of homing experiments and the predictions of random search that a very fair measure of quantitative agreement may be found. Now it must be emphasized that this agreement may not be interpreted as proving that random search is the mechanism of, or a large factor in, bird 'navigation', though the very fair quantitative agreement at all points open to direct investigation makes this seem possible. What it does show, however, is that these experiments are not at all susceptible of the interpretations which have sometimes been placed upon them; they are quite consistent with random search, and they cannot be made to yield any information at all about the existence of a real navigational ability. To demonstrate such an ability we should have to have very much more detailed information of the type provided in the experiments with gannets by Griffin & Hock (1949).

It is curious to reflect that the three starlings (Rüppell, 1937) and one coot (Rüppell & Schifferli, 1939), which were recovered on the homing flight very roughly on the direct line towards home, provide better evidence for the existence of a real navigational ability than all the returned birds put together; this evidence is very slight, and has practically no statistical significance. Nor may much significance be attached to the occasional remarkable feats of single birds such as the famous shearwater (Lack & Lockley, 1938) which homed from Venice to Skokholm.

The conclusion of this investigation must be that if birds possess any real navigational ability at all a demonstration of it does not seem likely to be forthcoming from experiments of this type, and that more sophisticated lines of approach are needed; examples of these are a close study of the behaviour of individual birds on release,* the plotting of a bird's actual course by aeroplane or other means, and the determination of the actual flying time as opposed to the total time between release and return (Wilkinson, 1950).

11. MIGRATION

Migration may properly be split up into two classes: the first contains that behaviour which is usually meant by the term migration, namely, a leaving of one more or less well-defined area by a bird or group of birds and the gaining of another, the two widely separated areas normally representing winter and summer quarters; this might be called anastrophic† migration; the second class is more properly a

* Such observations are often made as a matter of course in investigations such as those cited above. Usually the initial scattering is random; in some cases it is not, but as yet clear-cut evidence on initial orientation towards home in wild birds has not been forthcoming though it has been demonstrated by Matthews (1951) in the domestic pigeon.

† From *ἀναστρέφειν*, to retrace one's steps, and *διασπορά*, dispersion. I am grateful to Prof. A. J. Beattie and Mr H. J. Lloyd-Jones for the suggestion of these words.

scattering or dispersal in which birds leave their breeding quarters and do not gain well-defined winter quarters but rather wander over wide stretches of territory—usually sea—and reassemble at the breeding stations in the following season; this form of behaviour might be called diasporic* migration. Both anastrophic and diasporic migrations differ from homing in the essential particular that the bird transports itself and so always has the chance of determining its position by some sort of dead reckoning; there is not the initial uncertainty that confronts a bird artificially displaced in a homing experiment, and the call made on navigational ability is not of the same order. Though they remain remarkable feats of endurance and astonishing natural phenomena, there is no great difficulty in understanding the mechanism by which anastrophic migration flights are carried out when they are known to follow natural features such as coasts. There is probably some random element in the way the flight proceeds, but this is not very interesting in relation to the present problem. There remains a type of anastrophic migration flight which is more remarkable and which has often been adduced as evidence for the existence of true navigational ability; this comprises those flights which take place over large areas of open sea from point to point; the best-known example is that of the Pacific golden plover. Some of these plovers migrate to the Hawaiian Islands; the nearest point of their breeding territory is the Aleutians, about 2000 miles away to the north, and while suggestions have been made (Preston, 1949) that natural features may in fact exist to provide some sort of guide to the birds, the phenomenon remains one which merits discussion. As a basis of discussion we shall investigate the way in which random search may be combined with a measure of observation and instinctive behaviour. In point of fact, the problem is not as great as it has commonly been represented to be; the flight does not take place to one small isolated island, but rather to a very extended chain of islands, and the target for a plover leaving the Aleutians is not so much the island of Hawaii, as the 1600 mile-long archipelago of which Hawaii is a part. This simplifies enormously the problem posed by the flight, and shows that it will be very much easier than would be one which took place strictly between two widely separated small islands. We shall treat here this latter, quite hypothetical, problem, and the answers obtained will, therefore, be grossly pessimistic for the actual flight of the plover.

Imagine, then, two small islands, from one of which a bird starts to fly. The bird is imagined to have some instinctive tendency to fly in the general direction of the other island; we may not imagine it to have an instinct to fly directly there, for this would involve navigation of too high an order to be plausible and expose the bird too much to the influence of winds; we imagine rather that the bird may fly in any direction with equal probability at each turning point of its random flight, but that the lengths of the flights are no longer independent of their direction and tend to be longer when the flight direction happens to be towards home, a preference which may plausibly derive from the sun. The calculation may be performed for any pattern of flight lengths, but that shown in Fig. 14 has been chosen for discussion; the length of a flight in any direction is proportional to the length of the straight

* See note on previous page.

line from the cusp to the curve in that direction. (Any other pattern may be discussed; see the Appendix, §17.) The flight lengths are assumed rather small in the backwards direction, but large and comparable for a wide range of directions towards home. This means that the orienting influence is assumed to be only very feeble. Furthermore, the success of the homing flight does not depend very critically on the orientation of the pattern as a whole towards home—it may point wrongly by as much as 600–700 miles either way in the 2000 miles before a serious change occurs in the percentage return. We also assume that the bird can recognize to

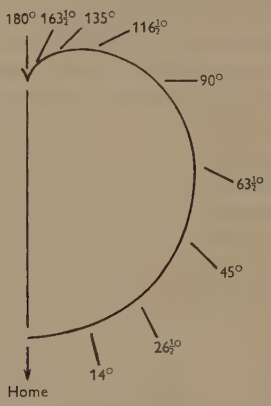

Fig. 14. Flight pattern assumed for discussion of trans-oceanic anastrophic migration; flight length is proportional to the distance of the curve from the cusp in any direction. (The flight pattern is symmetrical either side of the vertical line 180°—home.)

within a few hundred miles (again about 600–700) when it has reached the right latitude; this it might succeed in doing by many means. Our assumptions as to the bird's navigational abilities are very slight; a high degree of orientation is not demanded nor is great precision in recognizing the correct latitude. We finally assume that the island is visible from about 20 miles away, as it would be from an altitude of less than 400 ft.; we then need not assume that it has any size at all. Under these assumptions a crude mathematical treatment gives the simple result that the fraction of birds which reaches home is governed only by t_0/τ, where τ is twice the time which the birds would have taken had they flown in a straight line; it does not depend on the diffusivity κ.* Fig. 15 shows the percentage return as a function of this ratio t_0/τ (the analytical form is given in the Appendix, §17). It is seen that a 50% return may be achieved for $t_0/\tau = 4$. Now this calculation is pessimistic on several grounds as detailed above, and must be regarded as an estimate of a firm upper limit of the effort required. A more realistic treatment would

* Although the flight characteristics do not enter the result of this calculation, the value of L enters the allowable latitudes of 600–700 miles mentioned above; these are for an L of about 100 miles, but they change only as the square root of L; see the Appendix, §17.

certainly show the same percentage return for a very much smaller effort.* The acceptability of such an account of trans-oceanic migration must obviously rest on an estimate of t_0. Any account which leaves so little to the bird and so much to chance would demand a very long flight and the ability to rest on the sea; there seems to be no great difficulty in accepting this possibility.†

Diasporic migration may be similarly discussed, and the time required for the dispersed birds to reassemble at the breeding station may be estimated. We shall present explicitly the case in which the birds are dispersed uniformly over a strip of ocean of width a bounded on either side by straight coasts running, let us say,

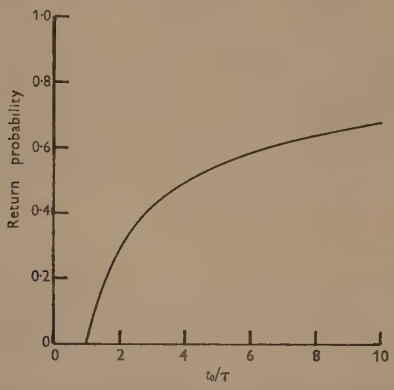

Fig. 15. Return probability as a function of search time (t_0) as t_0/τ for trans-oceanic anastrophic migration.

north and south, one of which may be recognized as unfriendly and constitutes a reflecting barrier, while the other contains home and retains the bird on its arrival there. These assumptions are probably reasonable: the bird may probably locate home in latitude to 500 or 600 miles by ecological clues; it then requires little searching, once a coast has been reached, to establish whether it contains home or not. If home is not associated with a coast or major land mass but rather with a small isolated island, then the situation approximates to the extreme case of anastrophic migration treated above, and the results of that discussion apply fairly well to the present problem. It would again be very reasonable to suppose some

* If it is indeed permissible to assume, as we do here, an orientation of the whole flight diagram towards home with an accuracy of about 20° either way, then most of the birds will, in fact, encounter some portion of the Hawaiian archipelago after time τ only, and so the effort required is only twice that appropriate to flight in a straight line. A rather sharper flight pattern could derive from crude solar navigation and with it successful homing to the archipelago with a still smaller effort. It is the writer's belief that this is probably the case for the Pacific golden plover.

† Very few golden plovers have ever been observed in flight over the ocean and none has ever been seen resting on the sea; this is not a strong argument against their so resting, as a flying bird is a very much more conspicuous object than a resting bird, and it is probable that resting would occur at night. There can be little doubt that the golden plover is capable of alighting on and taking off from open water when it is remembered that such unlikely species as the sand-martin (Stewart, 1950) and common buzzard (Stanes, 1950) appear to be capable of this feat.

rough knowledge of the direction of home such as may be given by the rising or setting sun, the original dispersion being probably not strictly random to begin with, but we present results gained without this aid and they are therefore pessimistic.

The random search for home begins at time zero, and we wish to know the percentage return as a function of time as in the last problem; this is shown in Fig. 16, where the percentage return is given as a function of $\kappa t/a^2$ (the analytical

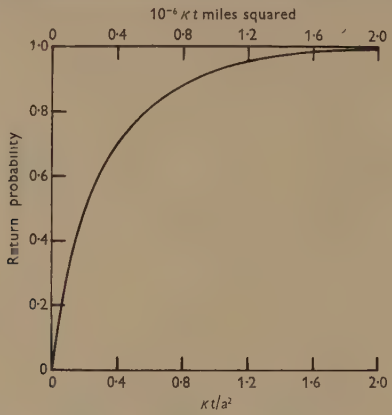

Fig. 16. Return probability in trans-oceanic diasporic migration in a bounded ocean of width a: lower abscissa scale, as a function of $\kappa t/a^2$; upper abscissa scale, as a function of $10^{-6}\kappa t$ in miles squared for $a = 1000$ miles.

form is given in the Appendix, §18); the upper abscissa scale shows the value of κt for $a = 1000$ miles, a figure appropriate to an ocean. We see that a 90% return is achieved after less than 30 days for $\kappa = 30{,}000$ miles squared per day, a plausible figure for sea birds derived from the discussion of homing experiments in §10; this means a real search time of 90 days if $f = 3$. This result seems to be very reasonable, and shows that diasporic migration presents no difficulty for the theory of random search.

12. CONCLUSION

It has been shown, in the preceding analysis, that the broad features of the experimental evidence on large-scale bird movements are exactly what would be expected on the basis of random search, both qualitatively and quantitatively: the four pieces of evidence, the percentage return versus distance, the speed versus distance, the 'maximum' time of return and the distribution of return times all fit together to form a unified picture within the framework of the theory of random search, and, furthermore, the flight constants which the theory finds it necessary to postulate to obtain this unification are not only inherently plausible but also agree well with the very scanty direct evidence on the detailed behaviour of birds released in unknown territory. In the face of this agreement of experiment

with the crudest possible theory, the application of which has usually adopted mathematical devices unfavourable to itself, it seems probable that we should assert that bird 'navigation' does indeed involve nothing but random search. But, as has been remarked above, we do not wish to do this but rather reverse the argument, and maintain that nothing about navigational ability can possibly be deduced from experiments of this type so long as they continue to give results such as those which have been forthcoming so far.

APPENDIX

13. *The mathematical approach*

The problem discussed here is appropriately known as the problem of random flights. It may be accurately solved without difficulty for some simple cases, but becomes excessively complicated for some of the geometries considered here. An alternative approach is permissible in the case when the distance of release from home is large compared with the step-length; we may then replace the random-flight problem by the one of diffusion of heat; the equation for the temperature ϕ,

$$\kappa \nabla^2 \phi = \frac{\partial \phi}{\partial t},$$

is solved for an appropriately situated instantaneous unit source of heat with appropriate boundary conditions ($\phi = 0$* for absorption by the known territory and $\partial\phi/\partial n = 0$ for reflexion by the natural boundary, n being the normal to the boundary). κ, the diffusivity, is then related to the constants of the random flight by $\kappa = \frac{1}{4} n \Lambda^2$, n being the number of flights per unit time and Λ^2 their mean square length. (This is the appropriate relation when the flights take place in a plane; if they take place in three dimensions then $\frac{1}{4}$ is replaced by $\frac{1}{6}$, and if in one, then $\frac{1}{4}$ is replaced by $\frac{1}{2}$; the latter condition obtains for strict coastwise homing.)

This approximate method of solving the problem gives quite good results, even though the distance of release from home approaches the step-length. As an illustration consider the case of homing along a line where the problem is easy to solve accurately. Consider all steps to be equal and of length unity, then if we start a distance X from the absorbing barrier, it may be shown that (see, for example, Chandrasekhar, 1943) the probability of arriving at the barrier at the Nth step is

$$P(N, X) = \frac{X(N-1)!}{[\frac{1}{2}(N-X)]\,![\frac{1}{2}(N+X)]!\,2^N},$$

with $N \geqslant X$; only odd or even values of N are taken according as X is odd or even. For $X \gg 1$ this is a very accurate approximation to the solution deriving from the equation of heat diffusion. Fig. 17 shows the probability of having arrived at the absorbing barrier by the Nth step (our percentage return) plotted as a function of N for $X = 1$ and 2. The step function follows the accurate expression $P(N, X)$, and

* This is not strictly the correct boundary condition; a more appropriate one would be that of boundary radiation into a vacuum (see Marshak (1947), for example), but the error introduced is small in our case.

the smooth curve shows the approximation afforded by the analogy to the diffusion of heat. It is seen that a good approximation is given even for the smallest possible values of X.

The solutions of the heat diffusion equation are, for the most part, elementary; an account is given, for example, by Carslaw & Jaeger (1948).

Some general results follow at once from the form of the equation: the first is that the probability of a bird being found at a given point and time is a function of κt only, and so the percentage return is a function of κt_0; secondly, the return probability per unit time is equal to κ times some function of κt.

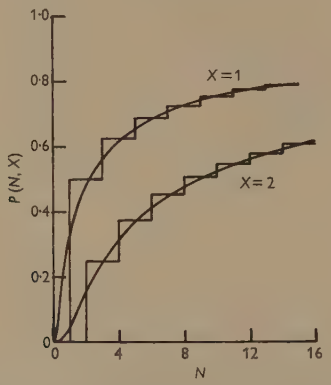

Fig. 17. Return probability $P(N, X)$ as a function of the number of steps taken (N) in the random walk problem in one dimension with absorbing barrier; X, the starting distance in step-lengths from the barrier, is shown on the curves. Smooth curve, approximation afforded by the diffusion of heat equation; step function, rigorous solution.

We shall summarize the results using the notations $p(t, x')$ for the probability of return per unit time at time t after release from a point x' distant from home, $M(t_0, x')$ for the probability of not having returned before time t_0 after release, and $\bar{s}(t_0, x')$ for the average speed of homing from x' up to time t_0. Then

$$M(t_0, x') = \int_{t_0}^{\infty} p(t, x')\, dt$$

and

$$\bar{s}(t_0, x') = \frac{\int_0^{t_0} \frac{x'}{t}\, p(t, x')\, dt}{1 - M(t_0, x')}.$$

14. *Homing along a semi-infinite coast*

All that we must do here is to satisfy the boundary condition $\phi = 0$ at $x = 0$

$$p(t, x') = \frac{x'}{2t\sqrt{(\pi\kappa t)}} \exp\left[-\frac{x'^2}{4\kappa t}\right],$$

$$M(t_0, x') = \operatorname{erf} \frac{x'}{2\sqrt{(\kappa t_0)}},$$

where
$$\operatorname{erf} x=\frac{2}{\sqrt{\pi}}\int_0^x e^{-z^2}\,dz,$$

$$\bar{s}(t_0, x')=\frac{2\kappa}{x'}\left\{\frac{\dfrac{x'}{\sqrt{(\pi\kappa t_0)}}\exp\left[-\dfrac{x'^2}{4\kappa t_0}\right]}{1-\operatorname{erf}\dfrac{x'}{2\sqrt{(\kappa t_0)}}}+1\right\}.$$

Simple forms will be found for M and $\bar{s}$ for limiting values of κt_0, but these are not very interesting in practice.

The results given under the imposition of intermediate familiar but not known territory follow from the form of the basic equation, and are quoted in §6.

15. *Homing along a bounded coast*

Here we have diffusion along a strip of length a and must satisfy the boundary conditions $\phi=0$ at $x=0$, $\partial\phi/\partial x=0$ at $x=a$.

$$p(t, x')=\frac{2\kappa}{a}\sum_1^\infty \alpha_n \sin\alpha_n x' \exp[-\kappa\alpha_n^2 t],$$

where
$$\alpha_n=\frac{\pi}{2a}(2n-1),$$

$$M(t_0, x')=\frac{2}{a}\sum_1^\infty \frac{\sin\alpha_n x'}{\alpha_n}\exp[-\kappa\alpha_n^2 t_0].$$

For sufficiently large values of the time we may use the first term only in the expansion, but this device is not so useful in this problem as in the next.

16. *Homing in a bounded continent*

This problem is considerably more complicated than the preceding ones, but allows of quite accurate approximations. It is of diffusion in a plane bounded by a reflecting circle of radius b and, concentric with it, an absorbing circle of radius a. Then rigorously

$$p(t, x')=\kappa\pi\sum_1^\infty \alpha_n^2\{J_1(b\alpha_n)\}^2\frac{Y_0(x'\alpha_n)\,J_0(a\alpha_n)-Y_0(a\alpha_n)\,J_0(x'\alpha_n)}{\{J_0(a\alpha_n)\}^2-\{J_1(b\alpha_n)\}^2}\exp[-\kappa\alpha_n^2 t],$$

where $\pm\alpha_n$ are the roots of the equation

$$J_0(a\alpha)\,Y_1(b\alpha)=Y_0(a\alpha)\,J_1(b\alpha),$$

and J and Y are Bessel functions of the first and second kind,

$$M(t_0, x')=\pi\sum_1^\infty\{J_1(b\alpha_n)\}^2\frac{Y_0(x'\alpha_n)\,J_0(a\alpha_n)-Y_0(a\alpha_n)\,J_0(x'\alpha_n)}{\{J_0(a\alpha_n)\}^2-\{J_1(b\alpha_n)\}^2}\exp[-\kappa\alpha_n^2 t_0].$$

Computation of these relations is facilitated by the observation that $b\gg a$; we may substitute for the Bessel functions the appropriate values for large and small argument and find, for instance, a good expression for the most important root,

$$\alpha_1=\frac{1}{b}\sqrt{\frac{2\left(2\log\frac{b}{a}-1-\sqrt{\left\{4\left(\log\frac{b}{a}\right)^2-8\log\frac{b}{a}+6\right\}}\right)}{\log\frac{b}{a}-\frac{5}{4}}}.$$

The second root may be approximated to within 2 or 3% by

$$\alpha_2=\frac{1}{b}\tan^{-1}\left\{\frac{\frac{2}{\pi}\left(\log\frac{4(b-a)}{3\pi\gamma a}\right)+1}{\frac{2}{\pi}\left(\log\frac{4(b-a)}{3\pi\gamma a}\right)-1}\right\},$$

where γ is Euler's constant 1·781..., after which successive roots have very nearly the uniform spacing, $\pi/(b-a)$. As the first root is much smaller than the spacing between roots the term for $n=1$ in the expansion rapidly becomes the dominant one, and a good expression for M, valid over practically all the range of κt_0 and b/a ratio considered here, is

$$M(t_0, x')=\frac{\frac{(b\alpha_1)^2}{2}\left(1-\frac{(b\alpha_1)^2}{8}\right)^2\left(\left(1-\frac{(x'\alpha_1)^2}{4}\right)\log\frac{x'}{a}+\frac{(x'\alpha_1)^2}{4}\right)}{1-\frac{(b\alpha_1)^2}{4}\left(1-\frac{(b\alpha_1)^2}{8}\right)^2}\exp\left[-\kappa\alpha_1^2 t_0\right].$$

To obtain a very crude measure of the variation of M with b/a we may drop all second-order terms when we find

$$M(t_0, x')\sim\frac{\log x'/a}{\log b/a}\exp\left[-\frac{2\kappa t_0}{b^2\log b/a}\right],$$

which demonstrates the slowness with which M depends on the assumed extent of the known territory, a dependence displayed more accurately in Fig. 12.

17. *Trans-oceanic anastrophic migration*

We use here the limiting form of the random flights problem with the step-length dependent on the phase; this has been given by Domb (1946) and Coulson (1947). Any polar diagram of step-length with direction may be dealt with, but the results become simple if we choose the form

$$D(\psi)=p+q\cos\psi,$$

where $D(\psi)$ is the step-length at an angle ψ between the flight and the direction of home. Fig 14, the pattern chosen for illustration in §11, has $p=q$. The centre of gravity of a large number of birds now drifts in the direction of home with a velocity $\frac{q}{2p}v$, and after N flights the probability distribution of birds about the centre of gravity of the bunch is

$$P(N, r)=\frac{2r}{NS}\exp\left[-\frac{r^2}{NS}\right],$$

where $P(N, r)\,dr$ is the probability of finding a bird between r and $r+dr$ from the centre of gravity of the bunch after N flights and $S=p^2+\frac{1}{4}q^2$.

We have assumed in §11 that the birds can recognize very crudely when they are in the latitude of home. This assumption may be applied rather roughly to the present calculation by arresting the diffusion characterized by $D(\psi)$ when the centre

of gravity of the bunch of birds has reached the latitude of home and then beginning a purely random search for home with the same value of the diffusivity κ: $4\kappa t = NS$.

We see, by comparing Figs. 2 and 11, that a fair representation of the percentage return of homing to a small circular home in two dimensions is given by the percentage return of homing from the same distance in one dimension (using $\kappa = \frac{1}{4}n\Lambda^2$ rather than $\frac{1}{2}n\Lambda^2$). We therefore take over this result (§ 14) and say that the probability of an unsuccessful migration flight is

$$M = \int_0^\infty P(N_0, r) \operatorname{erf} \frac{r}{2\sqrt{(\kappa t_0')}}\, dr.$$

$N_0 = 2B/q$, the number of flights before the centre of gravity reaches the latitude of home, B is the separation of the starting point and home, t_0' is the time of search remaining after N_0 flights have been taken:

$$M = \sqrt{\frac{1}{\frac{qt_0'v}{2Bp} + 1}}.$$

If we set $\tau = 2B/v$, twice the time required for flight in a straight line from the starting point to home, we have, for our special case $p = q$,

$$M = \sqrt{\frac{\tau}{t_0}},$$

where $t_0 = \tau + t_0'$ and is the maximum endurance in the sense used before.

The result is then independent of κ. κ must not be too small, however, or the bunch remains very concentrated and the result will then be sensitive to small errors in the orientation of the polar diagram towards home; this orientation may not be assumed to be very exact. For flights of the order $L = 100$ miles which we have found it necessary to assume to account for homing experiments, the angle $\sqrt{(2L/B)}$ subtended at the starting point by the half-width of the Gaussian distribution when the birds have reached the latitude of home is about 20° for the Pacific golden plovers; the orientation of the polar diagram towards home must then be accurate to this order. The spread at the latitude of home is itself of the order $\sqrt{(2LB)}$, or about 650 miles, and the birds must be capable of recognizing the latitude of home with about this accuracy.

18. *Diasporic migration*

The results of this problem may be derived independently but are obtainable by integrating the appropriate formulae of § 15 over all x' from 0 to a when we find that the fraction still searching at time t is

$$\frac{2}{a^2} \sum_1^\infty \frac{1}{\alpha_n^2} \exp\left[-\kappa\alpha_n^2 t\right],$$

the roots α_n are those of § 15.

SUMMARY

An analysis is presented of the results to be expected from experiments on the homing of wild birds if the only factor operating is random search. It is found that this model reproduces the experimental results and predicts values for the parameters involved in the theory which are inherently plausible and which are in quantitative accord with experimental evidence. Attention is paid to the dependence of percentage return on distance of release, to the dependence of the average speed of return on this distance, and to the distribution in time of the returns. These three sets of data form a coherent picture within the framework of the hypothesis of random search. Certain types of migration are also briefly considered.

It is not suggested that this investigation proves that random search is indeed the mechanism by which the homing of wild birds is accomplished, but it is submitted that the large-scale experiments of the type considered here are not susceptible of the interpretation that a true navigational ability is involved.

I would like to thank my wife for help with the numerical work that went into the preparation of the figures. I would like to thank also Dr W. H. Thorpe who first aroused my interest in the problems of bird navigation and Professor Sir Lawrence Bragg for his encouragement of this work.

REFERENCES

CARSLAW, H. S. & JAEGER, J. C. (1948). *Conduction of Heat in Solids*. Oxford.

CHANDRASEKHAR, S. (1943). Stochastic problems in physics and astronomy. *Rev. Mod. Phys.* **15**, 1–89.

CLAPARÈDE, E. (1903). La faculté d'orientation lointaine. *Arch. Psych. Geneva*, **2**, 133–80.

COULSON, C. A. (1947). Note on the random walk problem. *Proc. Camb. Phil. Soc.* **43**, 583–6.

DOMB, C. (1946). The resultant of a large number of events of random phase. *Proc. Camb. Phil. Soc.* **42**, 245–9.

GRIFFIN, D. R. (1940). Homing experiments with Leach's petrels. *Auk*, **57**, 61–74.

GRIFFIN, D. R. (1943). Homing experiments with herring gulls and common terns. *Bird Banding*, **14**, 7–33.

GRIFFIN, D. R. (1944), The sensory basis of bird navigation. *Quart. Rev. Biol.* **19**, 15–31.

GRIFFIN, D. R. & HOCK, R. J. (1949). Airplane observations of homing birds. *Ecology*, **30**, 176–98.

HODGE, C. F. (1894). The method of homing pigeons. *Pop. Sci. Mon.* **44**, 758–75.

LACK, D. & LOCKLEY, R. M. (1938). Skokholm Bird Observatory homing experiments. *Brit. Birds*, **31**, 242–8.

MARSHAK, R. E. (1947). Theory of the slowing down of neutrons by elastic collisions with atomic nuclei. *Rev. Mod. Phys.* **19**, 185–238.

MATTHEWS, G. V. T. (1948). Bird navigation. *New Nat.* **1**, 146–55.

MATTHEWS, G. V. T. (1951). The experimental investigation of navigation in homing pigeons. *J. Exp. Biol.* **28**, 508–36.

PRESTON, F. W. (1949). The Pacific flyway of the golden plover. *Auk*, **66**, 87–8.

RÜPPELL, W. (1935). Heimfindeversuche mit Staren. *J. Ornith.* **83**, 462–524.

RÜPPELL, W. (1936). Heimfindeversuche mit Staren und Schwalben. *J. Ornith.* **84**, 180–98.

RÜPPELL, W. (1937). Heimfindeversuche mit Staren, Rauchschwalben, Wendehälsen, Rotruck würgen und Habichten. *J. Ornith.* **85**, 120–35.

RÜPPELL, W. & SCHIFFERLI, A. (1939). Versuche über Winter-Ortstreue an *Larus ribidundus* und *Fulica atra* 1935. *J. Ornith.* **87**, 224–39.

STANES, R. G. F. (1950). Buzzard settling on the sea. *Brit. Birds*, **43**, 158.

STEWART, I. F. (1950). Sand-martins alighting on water. *Brit. Birds*, **43**, 121–2.

WILKINSON, D. H. (1950). Flight recorders. A technique for the study of bird navigation. *J. Exp. Biol.* **27**, 192–7.

THE THORACIC GLAND IN *RHODNIUS PROLIXUS* (HEMIPTERA) AND ITS ROLE IN MOULTING

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(With Six Text-figures)

It is now generally accepted that the periodic phases of growth in insects, which terminate in the deposition of a new cuticle and the casting of the old skin, are initiated by chemical changes in the circulating blood. For these growth-stimulating factors the general term 'moulting hormone' has been used (Wigglesworth, 1934), although it has been realized for some time that this hormone 'might ultimately prove to be made up of more than one constituent' (Wigglesworth, 1940) and that a succession of secretions might in fact be concerned (Williams, 1947; Wigglesworth, 1949).

The source of these active secretions appeared to differ in different insects. The original view of Kopeć (1922) that in Lepidoptera the secretion came from the brain was confirmed in *Rhodnius* (Wigglesworth, 1940) when it was shown that the neurosecretory cells of the pars intercerebralis were the apparent source of the 'moulting hormone'. But about the same time Fukuda (1940, 1941, 1944) found that in both the larva and pupa of the silkworm the prothoracic gland was the immediate source. So far as the Lepidoptera are concerned this controversy has been resolved by Williams (1947, 1948*a*), who has proved that secretions from the neurosecretory cells in the brain are necessary to activate the prothoracic glands.

In cyclorrhaphous Diptera it has long been recognized that the hormone causing pupation is produced in the ring gland of Weismann (Burtt, 1937, 1938; Hadorn, 1937) and almost certainly in the large lateral cells of this gland (Vogt, 1943) which are now commonly regarded as homologous with the 'pericardial glands' (or prothoracic glands) of other insects (Thomsen, 1941). It has recently been proved by Possompés (1950) that these lateral cells (called by him the 'peritracheal gland') are induced to secrete the pupation hormone by a factor liberated from the brain.

In *Dixippus*, 'ventral glands' and 'pericardial glands', again perhaps homologous with the prothoracic glands (Williams, 1948*b*, 1949; Pflugfelder, 1949), have been thought on histological grounds to be concerned in the control of moulting (Pflugfelder, 1947). Experiments on Odonata make it appear probable that the 'ventral glands' or 'intersegmental glands' are concerned (Deroux-Stralla, 1948). And in *Sialis* the moulting hormone appears to come from some centre in the thorax (Geigy & Ochsé, 1940). In *Gryllus* the brain is necessary for moulting (Sellier, 1949), but the histological changes in the prothoracic glands (Sellier, 1951) show that these also are probably concerned. In *Periplaneta* the immediate source of the 'moulting hormone' is the prothoracic gland (Bodenstein, 1953).

In the light of all these observations it has seemed probable for some time that a 'two-stage' control of moulting, neurosecretory cells activating a thoracic gland, might well prove general among insects. The purpose of this communication is to confirm the existence of such a system in *Rhodnius prolixus*.*

IMPLANTATION OF NEUROSECRETORY REGION OF THE BRAIN

It was shown earlier (Wigglesworth, 1940) that 4th-stage larvae of *Rhodnius* decapitated at 24 hr. after feeding can be induced to moult by implanting into the abdomen the dorsal region of the brain from larvae (either 4th stage or 5th stage) which have just passed the critical period, that is, at about 10 days after feeding in the 5th stage, about 7 days after feeding in the 4th stage (Fig. 1B). In the present series of experiments this result was obtained in sixteen (80%) out of twenty survivors.

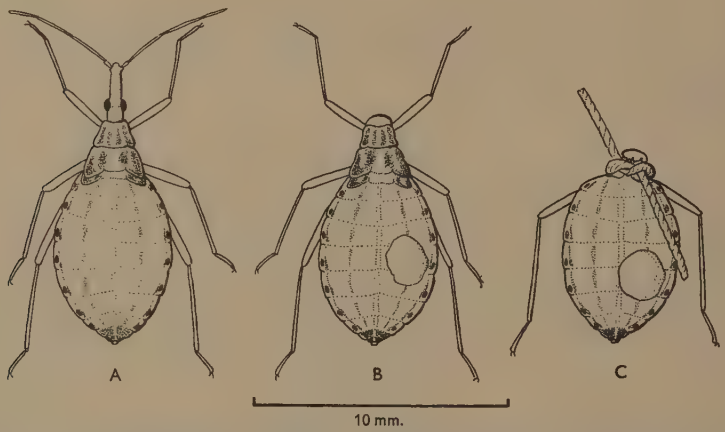

Fig. 1. A, normal 4th-stage larva of *Rhodnius*; B, the same decapitated with implant in abdomen; C, the same ligatured through metathorax with implant in the isolated abdomen.

Similar implantations were made into larvae 24 hr. after feeding, and the abdomen was then isolated by tying a firm ligature round the metathorax; the anterior part of the body was removed and the wound sealed with paraffin wax (Fig. 1C). The solated abdomen treated in this way fails to moult; there were no positive results in twenty-three experiments. Many of the body fragments remained alive for more than a month, but in none of them was there any sign of moulting even beginning. That is, there were none of the nuclear changes of 'activation' and mitosis which precede the deposition of a new cuticle (Wigglesworth, 1933).

It would appear, therefore, that the neurosecretory cells in the brain probably act, as in Lepidoptera, through the mediation of a secretory centre in the thorax.

* Preliminary note published (Wigglesworth, 1951).

THORACIC GLAND IN *RHODNIUS*

The presence of a thoracic gland in *Rhodnius* can be demonstrated as follows. The tergites of the mesothorax and prothorax of the 4th-stage or 5th-stage larva are removed by cutting along the margins and severing the muscles. The arrangement shown in Fig. 2A is then seen, with the cut muscles on either side, the green dorsal vessel in the mid-line and a mass of fat-body lying longitudinally below it.

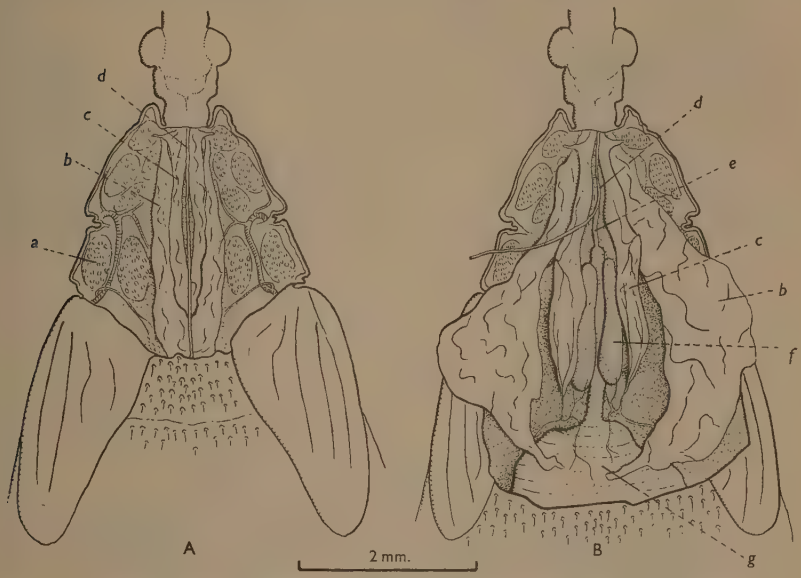

Fig. 2. A, 5th-stage larva of *Rhodnius* with dorsum of prothorax and mesothorax removed; B, the same with metathorax and part of abdominal tergites removed, and parts separated. *a*, cut muscles; *b*, outer lobe of thoracic fat-body; *c*, inner lobe of thoracic fat-body; *d*, dorsal vessel; *e*, oesophagus; *f*, salivary glands; *g*, stomach.

The dorsum of the metathorax and anterior segments of the abdomen are now cut away. The mass of fat-body can then be teased apart and is seen to consist of outer and inner lobes (Fig. 2B). The outer lobes are poorly supplied with tracheae and are extremely friable. The inner lobes are spindle-shaped, tapering to a point behind. They are richly supplied with tracheae which spread over their lateral aspects. The entire structure is much less friable than the outer lobe and can readily be dissected out without breaking up.

The tracheal supply comes from the anterior extremity, from the ventro-lateral aspect about one-third of the distance from the neck, and particularly from the tapering posterior extremity which is tied by tracheae to the salivary glands, to the narrow commencement of the mid-gut and to the 'stomach'. The 'retort-shaped' organs of the stylets extend for a variable distance into the base of the inner lobes.

It is not possible to recognize the thoracic gland in such dissections; but if the inner lobe of the thoracic fat-body is isolated and stained with haematoxylin it can be seen that there is a network of cells with very large nuclei spread out over its

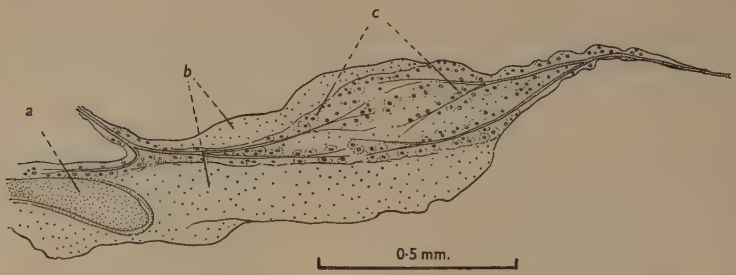

Fig. 3. Inner lobe of thoracic fat-body removed from the right side and seen from the dorso-latera aspect. *a*, retortiform organ; *b*, fat-body; *c*, thoracic gland in form of single layer of scattered cells well supplied by tracheae.

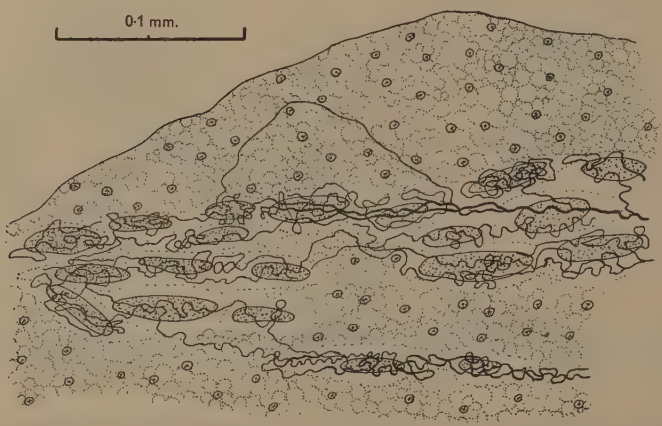

Fig. 4. Detail of part of fat-body and thoracic gland showing rich supply of tracheoles (injected cobalt sulphide) surrounding the gland cells.

lateral aspect (Fig. 3). This is clearly the thoracic gland; it has characters resembling those of the prothoracic gland in Lepidoptera (Williams, 1948*b*). It is abundantly supplied with tracheae; the rich tracheal network is limited to the superficial layer of the inner lobe and to those parts of it on which the thoracic gland is spread. Fig. 4 shows a part of the gland stained with haematoxylin after injection of the tracheal system with cobalt sulphide (Wigglesworth, 1950). The extremely rich tracheal supply to the gland is in striking contrast with the very sparse supply to the cells of the fat-body.

(During moulting the 'retortiform organs' of the developing stylets extend backwards and enlarge until the inner lobes of the fat-body become thin-walled sacs in which they are enclosed. The retortiform organs will thus benefit from the rich tracheal supply to the surface of the lobe; but it seems clear that this supply is connected primarily with the thoracic gland, for it runs only to that part of the surface that is occupied by the gland, and this comprises no more than one-sixth or less of the total surface when the lobe is fully distended.)

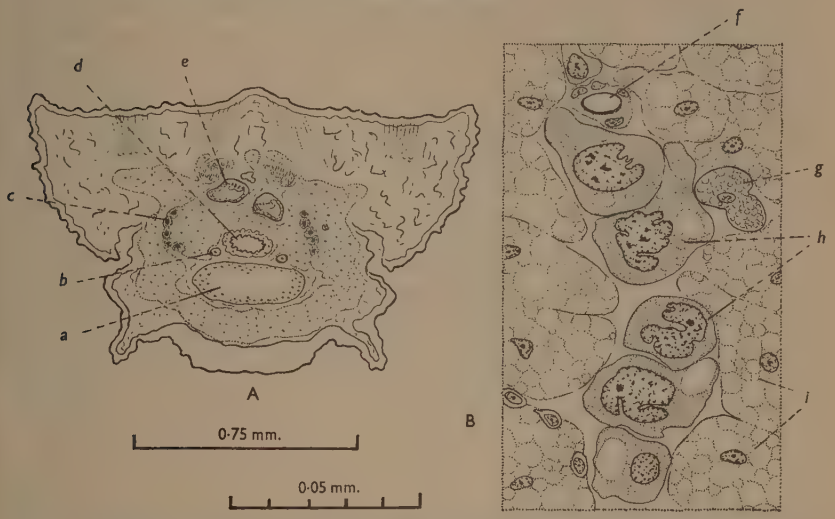

Fig. 5. A, transverse section of thorax of 4th-stage larva at level of mesothoracic spiracles at 9 days after feeding; B, detail of the same showing the thoracic gland cells. *a*, nerve cord; *b*, duct of salivary gland; *c*, thoracic gland lying on outer surface of the inner lobe of the fat-body; *d*, oesophagus; *e*, retortiform organ; *f*, trachea; *g*, muscle; *h*, enlarged cells of thoracic gland with lobulated nuclei; *i*, fat-body cells.

Fig. 5A shows a transverse section through the thorax of the 4th-stage larva at the level of the mesothoracic spiracles to show the relation of the thoracic gland cells, deeply embedded in the fat-body, to the other organs. Fig. 5B shows the detail of the gland cells.

The prothoracic gland in Lepidoptera is well supplied with nerves (Lee, 1948; Williams, 1948*b*), and that of the cockroach *Leucophaea* is innervated from the prothoracic ganglion (Scharrer, 1948*a*). No nerves could be seen in sections or dissections of the thoracic gland in *Rhodnius*. A number of 4th-stage and 5th-stage larvae were injected with methylene blue or methylene blue and rongalite, but even where the nerves to the adjacent muscles, to the salivary glands and to the gut were well stained no trace of any nerve supply to the thoracic gland could be seen. It is still possible, however, that the nerves have been overlooked.

CHANGES IN THE THORACIC GLAND DURING MOULTING AND METAMORPHOSIS

The thoracic gland has been studied in sections and in whole mounts throughout the moulting cycle in 4th-stage and 5th-stage larvae. In the unfed larva (Fig. 6A) the cells are shrunken and pale-staining, the nuclei are elongated and smooth in outline, and there are a few scattered haemocytes present.

After feeding, the cytoplasm gradually increases in extent and in depth of staining, and the nuclei become greatly enlarged and lobulated, and great numbers of haemocytes are applied to the surface of the gland. These changes reach their peak during

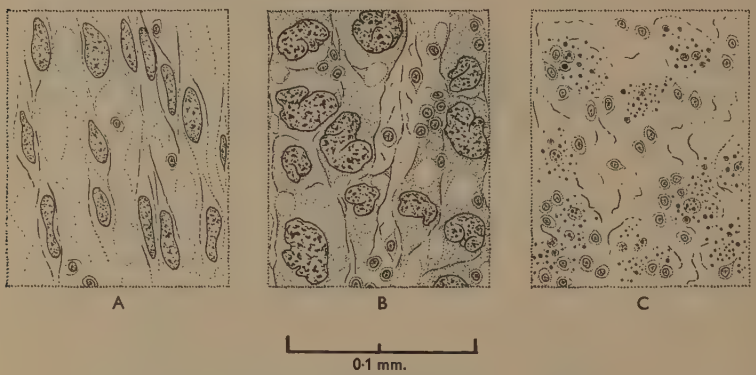

Fig .6. A, part of thoracic gland in unfed 5th-stage larva showing a few haemocytes; B, the same in 5th-stage larva at 10 days after feeding showing increased numbers of haemocytes; C, the same in adult *Rhodnius* one day after moulting showing numerous haemocytes around the disintegrating nuclei.

the 'critical period' 7–12 days after feeding (Fig. 6B). Thereafter the cells and nuclei slowly diminish in size, and by the time of moulting they have almost reverted to the resting condition.

When the 4th-stage larva has moulted to the 5th stage the thoracic gland remains in the resting condition until the new moulting cycle is initiated by a meal of blood. In the adult insect, on the other hand, the gland cells quickly break down and disappear.

In the newly moulted adult the cells are shrunken but the nuclei are intact. At 24 hr. after moulting almost no healthy nuclei remain; everywhere they are breaking down with the liberation of innumerable chromatic droplets (just like those in the epidermis during moulting (Wigglesworth, 1942)) and among them are numerous haemocytes (Fig. 6C). At 2 days after moulting the chromatic droplets are reduced in number; and at 3 days after moulting they have virtually disappeared, no vestiges of the thoracic gland remain, and the haemocytes are relatively sparse.

IMPLANTATION OF THE THORACIC GLAND

Implantation of the fat-body, removed from *Rhodnius* larvae just after the critical period, into larvae decapitated at 24 hr. after feeding, will not induce moulting (Wigglesworth, 1940). This has been confirmed. But if the inner lobes of the thoracic fat-body carrying the thoracic gland are removed from 4th-stage or 5th-stage larvae which have just passed the critical period and implanted into the abdomen of 4th-stage larvae decapitated at 24 hr., these are caused to moult. In the first trial there were six positive results in eighteen experiments.

If these lobes are implanted into larvae ligatured through the metathorax (Fig. 1 C) the isolated abdomen is likewise caused to moult within about 1 month. There were seven positive results among thirteen survivors.

As in Lepidoptera the thoracic gland appears to be the source of the secretion immediately responsible for initiating growth and moulting. Implantation of the dorsum of the brain is effective in inducing moulting only if thoracic glands are present.

Adult *Rhodnius* can be made to moult by joining them to 5th-stage larvae which have passed the critical period (Wigglesworth, 1940). If the above interpretation is correct the adult should not be caused to moult by implantation of the brain, since it lacks thoracic glands. That has proved to be the case: ten adult female *Rhodnius* (about 2 weeks after moulting, 24 hr. after feeding) each received implants of two brains removed from 5th-stage larvae 10 days after feeding. None moulted; egg development and oviposition occurred normally.

On the other hand, of ten adult *Rhodnius* each receiving implants of thoracic glands removed from 5th-stage larvae 9 days after feeding, four moulted; that is, they developed a new cuticle preliminary to moulting. This new cuticle was in all cases exceedingly thin and delicate; but this was probably because the development of eggs in the females and of the accessory glands in the males was not inhibited by the process of moulting; consequently, the food material which might have been used for cuticle formation was diverted for reproductive purposes.

Another series of eleven adults into which the thoracic glands from 5th-stage larvae had been implanted were therefore decapitated in order to suppress the activity of the reproductive system (Wigglesworth, 1936). Of these, four moulted and three of them laid down cuticles of normal thickness.

In order to see whether secretion from the brain of the adult *Rhodnius* would serve to activate the thoracic gland if such were present, twenty adult *Rhodnius* of mixed sexes, about 2 weeks after moulting and 24 hr. after feeding, received implants of thoracic glands from *unfed* 5th-stage larvae. Mating and oviposition occurred normally; none showed any signs of moulting. It would appear, therefore, that the absence of moulting in the normal adult *Rhodnius* is due both to the absence of the thoracic glands and the failure of the appropriate activity in the secretory cells of the brain.

It is to be noted that when the thoracic glands of the moulting 5th-stage larva are implanted into the adult both moulting and egg development take place. On the

other hand, it was previously observed (Wigglesworth, 1940) that when the adult female *Rhodnius* is caused to moult by joining it to two moulting 5th-stage and two moulting 4th-stage larvae with brain, corpus cardiacum and corpus allatum intact, the formation of eggs was arrested.

This result has been confirmed in further experiments in which nine adult females with their heads intact were caused to moult by joining to each of them two 5th-stage larvae with the tip of the head removed at 10 days after feeding. None of them showed any egg development, in spite of the fact that 'juvenile hormone' was being secreted by the corpus allatum of the adult females themselves (Wigglesworth, 1948), and caused the 5th-stage larvae to develop partial larval characters when they moulted. This suppression of egg development in the presence of the intact brain and retrocerebral organs of the 5th-stage larva is at present unexplained.

THORACIC GLAND IN OTHER HEMIPTERA

Scharrer (1948*a*) and Pflugfelder (1949) have suggested that the 'tracheal organs' described by Hamilton (1931) (among others) in *Nepa* may correspond with the prothoracic glands of other insects. But these are usually regarded as vestigial flight muscles; they do not consist of glandular cells; and they persist in the adult. A re-examination of *Nepa* has revealed the presence of a very distinctive glandular organ in the thorax of the larva, not mentioned by Hamilton, which is completely absent in the adult. This organ, and the thoracic glands in certain other Hemiptera, will be described elsewhere.

The only other reference to thoracic glands in Hemiptera is by Williams (1949), who records that they have been observed by Edwards (unpublished) in the Lygaeid *Oncopeltus*.

DISCUSSION

In the light of the experiments described in this paper and the accumulated evidence from other groups of insects as set out in the introduction, it appears that in many, perhaps all insects, the 'moulting hormone' is composite and consists at least of (*a*) an 'activating factor' from secretory cells in the brain and (*b*) a 'moulting factor' from the thoracic gland (prothoracic gland, ventral gland, pericardial gland, peritracheal gland).

No generally accepted terminology for these factors yet exists. In earlier papers dealing with *Rhodnius* the complex has been referred to as the 'moulting hormone' because the terminal event in the process of growth which it brings about is the moulting of the old skin. Scharrer (1948*a*, *b*) refers to it as the 'growth and differentiation hormone', since she conceives this complex as being responsible for the differentiation of imaginal (or pupal) characters. The same point of view is adopted by Piepho (1942).

Certainly these secretions are 'growth hormones'—though I am inclined to use this expression as a comprehensive term embracing all those humoral factors concerned in regulating growth ('activating factor', 'moulting factor', 'juvenile' or 'inhibitory' hormone, etc.).

On the other hand, it is perhaps misleading to describe this complex as a 'differentiation hormone'. Differentiation is the realization of potential capacities for growth hitherto latent in the different body cells. Looked at from the present point of view, insects have dual potentialities: larval and imaginal. The choice between these is controlled by the amount of the juvenile hormone secreted by the corpus allatum. The insect cannot moult without differentiating either in the larval or the imaginal direction. It is characteristic of the hormone from the thoracic gland that it merely initiates growth and moulting—the type of differentiation is determined by other factors, partly humoral, partly inherent in the cells.*

One further point which calls for comment is the very rapid disintegration of the thoracic gland immediately the 5th-stage *Rhodnius* becomes adult. There must be some abrupt change in the internal environment which is responsible for this breakdown, but the cause of it has not yet been discovered. The similar though more gradual breakdown of the prothoracic gland in the adult *Periplaneta* has been shown by Bodenstein (1953) to depend upon a change in the corpus cardiacum of the adult.

SUMMARY

The 'moulting hormone' in *Rhodnius* is composite. The factor secreted in the dorsum of the brain activates a gland in the thorax which then produces the factor initiating growth and moulting. Implantation of the thoracic gland will induce moulting in the isolated abdomen; implantation of the brain is effective only if the thorax is intact.

This system agrees with that described in Lepidoptera and Diptera and is probably widespread in insects.

The thoracic gland in *Rhodnius* consists of a loose network of very large cells, richly supplied with tracheae, spread as a single diffuse layer over the surface of the inner lobes of the thoracic fat-body. These cells go through a cycle of secretory activity which reaches its peak during the critical period. They break down and disappear within 2 days after the insect becomes adult.

The adult *Rhodnius* is caused to moult by implantation of the thoracic gland from a moulting larva; it is not caused to moult by implantation of the brain.

REFERENCES

Bodenstein D. (1953). Endocrine control of metamorphosis. *Proc. IXth Int. Congr. Entom.* Amsterdam, 1951 (in the Press).†

Burtt, E. T. (1937). On the corpora allata of Dipterous insects. *Proc. Roy. Soc.* B, **124**, 13–23.

Burtt, E. T. (1938). On the corpora allata of Dipterous insects. II. *Proc. Roy. Soc.* B, **126**, 210–23.

Deroux-Stralla, D. (1948). Recherches expérimentales sur le rôle des 'glandes ventrales' dans la mue et la métamorphose, chez *Aeschna cyanea* Müll. (Odonata). *C.R. Acad. Sci., Paris*, **227**, 1277–8.

Fukuda, S. (1940). Induction of pupation in silkworm by transplanting the prothoracic gland. *Proc. Imp. Acad. Japan*, **16**, 414–6.

* Novak (1951) pictures differentiation as being controlled by the relative concentration in the different regions of the body of another (intracellular) hormone which he terms the 'gradient factor'. This hypothesis awaits further development.

† Quoted by kind permission of Dr Bodenstein.

FUKUDA, S. (1941). Role of the prothoracic gland in differentiation of the imaginal characters in the silkworm pupa. *Annot. zool. jap.* **20**, 9–13.

FUKUDA, S. (1944). The hormonal mechanism of larval moulting and metamorphosis in the silkworm. *J. Fac. Sci. Tokyo Univ.* sec. IV, **6**, 477–532.

GEIGY, R. & OCHSÉ, W. (1940). Versuche über die inneren Faktoren der Verpuppung bei *Sialis lutaria* L. *Rev. suisse Zool.* **47**, 225–41.

HADORN, E. (1937). Hormonale Kontrolle der Puppariumbildung bei Fliegen. *Naturwissenschaften*, **25**, 681–2.

HAMILTON, M. A. (1931). The morphology of the water-scorpion *Nepa cinerea* L. (Rhynchota). *Proc. Zool. Soc. Lond.* pp. 1068–1136.

KOPEĆ, S. (1922). Studies on the necessity of the brain for the inception of insect metamorphosis. *Biol. Bull. Woods Hole*, **42**, 322–42.

LEE, H. T. Y. (1948). A comparative morphological study of the prothoracic glandular bands of some Lepidopterous larvae with special reference to their innervation. *Ann. Ent. Soc. Amer.* **41**, 200–5.

NOVAK, V. J. A. (1951). New aspects of the metamorphosis of insects. *Nature, Lond.*, **167**, 132–3.

PFLUGFELDER, O. (1947). Über die Ventraldrüsen und einige andere inkretorische Organe des Insektenkopfes. *Biol. Zbl.* **66**, 211–35.

PFLUGFELDER, O. (1949). Die Funktion der Pericardialdrüsen der Insekten. *Verh. dtsch. zool. Ges.* pp. 169–73.

PIEPHO, H. (1942). Untersuchungen zur Entwicklungsphysiologie der Insektenmetamorphose. *Roux Arch. Entw. Mech. Organ.* **141**, 500–83.

POSSOMPÉS, B. (1950). Rôle de cerveau au cours de la métamorphose de *Calliphora erythrocephala* Meig. *C.R. Acad. Sci., Paris*, **231**, 594–6.

SCHARRER, B. (1948*a*), The prothoracic glands of *Leucophaea maderae* (Orthoptera). *Biol. Bull. Woods Hole*, **95**, 186–98.

SCHARRER, B. (1948*b*). Hormones in insects. *The Hormones* (G. Pincus and K. V. Thimann, edd.), **1**, 121–58.

SELLIER, R. (1949). Diapause larvaire et macroptérisme chez *Gryllus campestris* (Orth.). *C.R. Acad. Sci., Paris*, **228**, 2055–6.

SELLIER, R. (1951). La glande prothoracique des gryllides. *Arch. Zool. exp. gén.* **88** (Notes et Rev.), 61–72.

THOMSEN, E. (1941). Ringdrüse und corpus allatum bei Musciden. *Naturwissenschaften*, **29**, 605–6.

VOGT, M. (1943). Zur Produktion und Bedeutung metamorphosefördernder Hormone während der Larvenentwicklung von *Drosophila*. *Biol. Zbl.* **63**, 395–446.

WIGGLESWORTH, V. B. (1933). The physiology of the cuticle and of ecdysis in *Rhodnius prolixus*. *Quart. J. Micr. Sci.* **76**, 269–318.

WIGGLESWORTH, V. B. (1934). The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and metamorphosis. *Quart. J. Micr. Sci.* **77**, 191–222.

WIGGLESWORTH, V. B. (1936). The functions of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. Micr. Sci.* **79**, 91–121.

WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17**, 201–22.

WIGGLESWORTH, V. B. (1942). The significance of 'chromatic droplets' in the growth of insects. *Quart. J. Micr. Sci.* **83**, 141–52.

WIGGLESWORTH, V. B. (1948). The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **25**, 1–14.

WIGGLESWORTH, V. B. (1949). Rapport général sur l'endocrinologie des insectes. *Bull. Biol.*, Suppl., **33**, 174–6.

WIGGLESWORTH, V. B. (1950). A new method for injecting the tracheae and tracheoles of insects. *Quart. J. Micr. Sci.* **91**, 217–24.

WIGGLESWORTH, V. B. (1951). Source of moulting hormone in *Rhodnius*. *Nature, Lond.*, **168**, 558.

WILLIAMS, C. M. (1947). Physiology of insect diapause. ii. Interaction between the pupal brain and prothoracic glands in the metamorphosis of the giant silkworm, *Platysamia cecropia*. *Biol. Bull. Woods Hole*, **93**, 89–98.

WILLIAMS, C. M. (1948*a*). Extrinsic control of morphogenesis as illustrated in the metamorphosis of insects. *Growth Symp.* **12**, 61–74.

WILLIAMS, C. M. (1948*b*). Physiology of insect diapause. iii. The prothoracic glands in the Cecropia silkworm. *Biol. Bull. Woods Hole*, **94**, 60–5.

WILLIAMS, C. M. (1949). The prothoracic glands of insects in retrospect and in prospect. *Biol. Bull. Woods Hole*, **97**, 111–14.

THE EVAPORATION OF WATER FROM SPIDERS

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(With Two Text-figures)

INTRODUCTION

Insects are generally recognized as the most successful invertebrate animals on land, but judged by the number of individuals and species, or by the range of habitats occupied, spiders are not far behind. Efficient mechanisms for water conservation are essential for the success of small animals on land, and, in particular, loss of water by evaporation must be restricted. It is well known that in insects evaporation is limited at lower temperatures by a relatively impermeable wax layer which undergoes, at a critical temperature characteristic for each species, a change in physical structure which permits a much higher rate of evaporation above this temperature.

The only information available concerning the effect of temperature on the evaporation of water from spiders is that obtained by Palmgren (1939). Using *Dolomedes fimbriatus*, he found that evaporation rises steadily with temperature, but he did not work at sufficiently high temperatures to demonstrate the presence or absence of a critical point. The present work was therefore undertaken primarily to find whether the integument of spiders behaves in the same way as that of insects, so far as restraint of evaporation is concerned, and subsidiary problems concerned the significance in this respect of the respiratory organs—the lung-book and 'tracheae'.

MATERIAL

Much of the work was carried out on the wolf spider, *Lycosa amentata* (Clerk), which was collected from the University grounds. Other species used for comparison were *Meta segmentata* Clerk, *Zilla atrica* (Koch) and *Z. x-notata* Clerk (all belonging to the family Epeiridae) which were also collected from the University grounds; and *Tegenaria derhami* Scopoli, an Agelinid, obtained from outhouses in the Birmingham district and from Surrey.

The lycosids were kept on sand, each in a separate tube to prevent cannibalism, at a relative humidity of about 75%. They were fed on *Drosophila*, and survived indefinitely. The other species were kept in similar conditions, but they were used soon after collection and were not fed.

ANATOMY OF THE RESPIRATORY ORGANS

Before attempting to measure the rate of evaporation from the integument it was necessary to know the extent to which water evaporates from the respiratory surfaces. The only information is again due to Palmgren (1939), who found that

at 17° C. and 60% R.H. only 0·01 mg. water per hour was evaporated from the lung-books as compared with 0·78 mg. from the rest of the integument, and he considered it unnecessary to seal the lung-book spiracles when measuring evaporation from the integument. He considered the amount of water evaporated from the four unbranched tracheae to be insignificant, and they were also left unsealed.

Since the number and arrangement of the lung-books varies from one species of spider to another, it was decided to study the anatomy of these organs in the species to be used in the present work, and then, should it appear necessary, to attempt to

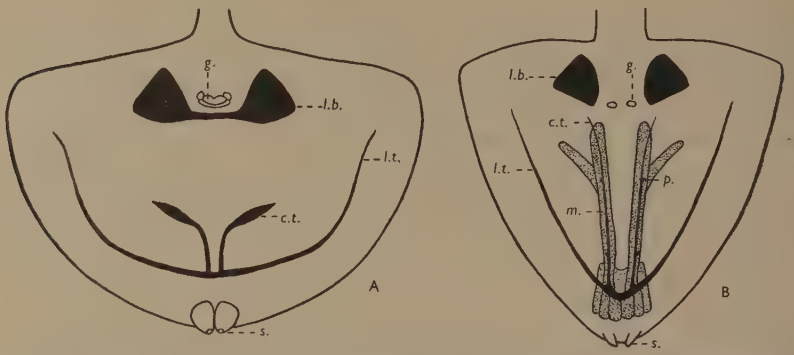

Fig. 1. Arrangement of the tracheae in (A) *Zilla atrica*, and (B) *Lycosa amentata*. Diagrammatic. *c.t.*, central trachea; *g.*, genital apparatus; *l.b.*, lung-book; *l.t.*, lateral trachea; *m.*, ventral longitudinal muscle; *p.*, attachment process; *s.*, spinerets.

measure the evaporation of water from them. In part this was a repetition of the work of Lamy (1902) and of Remy (1925) but their accounts do not contain all the information required. There is no published description of the tracheae of *Lycosa amentata*, so these will be described in some detail.

Advantage was taken of the technique developed by Wigglesworth (1950) for injecting the tracheae of insects with cobalt sulphide. This proved very successful with spiders, and permanent preparations were made of injected and bleached specimens of all the species studied. Fig. 1, which shows diagrammatically the arrangement of the tracheae in *Lycosa* and *Zilla*, is based on such preparations. (In life the tracheae follow an irregular course, moving with the contents of the abdomen.) The tracheae of *Meta* and *Tegenaria* are similar to those of *Lycosa*.

There are two pairs of unbranched tracheae in *Lycosa*, which run forwards from a vestibule, and the latter opens by a small spiracle just anterior to the spinning plate on the ventral surface. In a spider whose total length is 7·0 mm., each lateral trachea is about 2·4 mm. long, with a diameter at the base of 0·15 mm., tapering gradually to the tip, which is anchored to the body wall in the antero-lateral region of the abdomen. The terminal ninth of these tracheae is colourless after injection, and is therefore presumably solid; the rest of their length is filled with a black precipitate of cobalt sulphide, and is hollow. The central tracheae are about 1·7 mm.

long, they leave the antero-lateral corners of the vestibule and run straight forwards along the ventral longitudinal muscles. At a point about one-fifth of the total length away from the vestibule, each trachea bears a small process which serves to anchor it to the underlying muscle, and there are similar processes rather more than half-way along, providing attachment to a dorso-ventral muscle. Beyond this point the tracheae are free; they are black up to the tips in injected specimens and therefore hollow. As pointed out earlier by Remy and others, the tracheae are ectodermal invaginations, they are lined with cuticle, and their lumen is kept open by a number of small chitinous spines. The chitinous layer is surrounded by typical epidermal cells. Examination of sections cut at 10μ confirmed these observations.

The lung-books of many spiders have been adequately described (e.g. Kastner, 1924, 1929), and will not be referred to in detail here. In cobalt-sulphide injected specimens they appear intensely black, and their anatomy is best studied in fresh material with a stereoscopic microscope. Each lung-book in *L. amentata* contains from 30 to 55 triangular lamellae. An estimate of the total respiratory surface in this species was made by means of camera lucida drawings of a sample of five lamellae from each of five spiders. The mean area per spider was 9·0 mm.^{2}, though there was much variation between one spider and another owing to differences in size and in numbers of lamellae present. The highest value recorded was 12·5 and the lowest 6·7 mm^2. An estimate of the surface area of the tracheae in the same species was obtained by flattening and making camera lucida drawings as before. The mean figure was 0·30 mm^2. and the extremes were 0·33 and 0·25 mm^2. It is clear from these figures that the respiratory surface of the lung-books is approximately thirty times as great as that of the tracheae.

RESPIRATORY FUNCTION OF THE TRACHEAE

Remy (1925) used indigo blue injected in the reduced, colourless form, to demonstrate the respiratory function of the tracheae in a number of spiders. This was repeated on *Lycosa amentata*. The reduced material was injected into the abdomen and the animal was dissected under oxygen-free saline after 3 min. Both the lung-books and the tracheae were intensely blue, demonstrating the presence of free oxygen in those areas. It is clear, therefore, that oxygen does enter the body of the animal via the tracheae, and an attempt was then made to measure the amount absorbed in this way and to compare it with the amount absorbed via the lung-books.

A standard Warburg manometer apparatus was used, running at 30° C. Spiders were exposed for periods of 5 hr., one in each flask of the apparatus, and enclosed in a small muslin sac to prevent undue movement. Twelve spiders were used in each of three groups: in the first, the animals were intact; in the second, lung-book spiracles were blocked with celloidin, and in the third the openings to the tracheae were similarly blocked. Animals in the second group died after about 4 hr. Readings were made at the end of each hour, and the results were expressed as the volume of oxygen absorbed in microlitres per mg. during the whole 5-hr. period. This figure was calculated separately for each spider and a mean was then obtained.

The results (means and standard deviations) were as follows:

Intact spiders	Lung-books blocked	Tracheae blocked
2·9 (0·5)	0·0 (0·0)	3·3 (0·7)

Clearly there was a great deal of variability, and there is no significant difference between the means for intact spiders and those with the tracheae blocked. Two related facts, however, emerge—first, that there is no *measurable* uptake of oxygen through the tracheae; and secondly, that tracheal respiration, if it occurs at all, is insufficient to keep the animals alive. Now the respiratory surface offered by the tracheae has been shown above to be one-thirtieth of that of the lung-books. If the assumption is made that the permeability to oxygen of the two surfaces is similar, then one-thirtieth of 0·6 μl. of oxygen per mg. of spider will enter through the tracheae in 1 hr. A spider weighs about 20 mg., so that a total of 0·4 μl. per hour will enter the tracheae, and this amount is too small to give a significant reading on the manometer employed. The results obtained with this apparatus are not, therefore, inconsistent with those obtained by means of the indigo blue technique.

THE EFFECT OF CARBON DIOXIDE ON EVAPORATION FROM THE LUNG-BOOKS

In view of the very small tracheal surface demonstrated above it was not considered necessary to block their spiracles when measuring evaporation. The lung-books, however, present a much greater surface, and evaporation from them might be expected to form a significant proportion of the total. In insects, if the spiracles are kept open by exposing the animals to CO_2 in air, evaporation is greatly increased. It is also known that the spiracles to the lung-books in spiders are nearly closed in the resting animal and may be caused to open by exposure to CO_2 and air mixtures (Hazelhoff, 1926*a*, *b*). The following experiment was carried out to find whether such enforced opening would lead to an increased rate of evaporation from *Lycosa*.

Spiders were exposed in test tubes. Each tube was graduated into ten parts, inverted over mercury and the pressure equalized, so that mercury occupied one-tenth of the volume. CO_2 was allowed to bubble in, displacing the mercury, and each tube was then sealed with a vaselined glass slip. Several tubes were checked after preparation in this way by inverting them over KOH, when the latter rose accurately to the 10% mark. The method is simple, and critical accuracy is clearly unnecessary in an experiment of this sort.

Each tube also contained, at the bottom, a small muslin bag of calcium chloride. This was held in place by a tightly fitting gauze plate which also served as a platform for the spider. Four series of spiders, each of eight individuals, were used: in two series the animals were alive, in the others they had been killed by exposure to KCN. Both living and dead spiders were exposed either to air or to 10% CO_2 in air. Loss in weight during 24-hr. exposures was taken as a measure of the water evaporated and was expressed as a percentage of the original weight of the spider. The results (means and standard deviations) were as follows:

	Alive	Dead
In air	16% (5·2%)	19% (5·5%)
In 10% CO_2	23% (5·7%)	18% (3·8%)

There is clearly a good deal of variability, but there is a significant difference between the means for living spiders in air and in CO_2 ($p < 0{\cdot}05$). There is no significant effect of CO_2 upon the rate of evaporation from dead spiders. There is a suggestion that dead spiders lose water more rapidly than living ones in air; the difference is not statistically significant, but it will be discussed further below (p. 580). The fact that living spiders in CO_2 lose more water than dead ones is explicable if the spiracles of dead spiders are not caused to open wide by CO_2.

THE EFFECT OF TEMPERATURE UPON EVAPORATION

The results obtained in the last experiment indicate that the rate of evaporation from living spiders is increased by nearly half if the spiracles are kept open. The amount of evaporation from normal (partially closed) spiracles, as compared with the rest of the integument, is still uncertain, and so is the effect of temperature upon total and spiracular evaporation. The following experiments were designed to provide this information.

(*a*) *Experiments with* Lycosa amentata

Spiders were exposed six at a time, but separated from one another, to a slowly moving stream of dry air (*ca.* 5 cm./sec.) at constant temperatures for periods of 15 min. The apparatus used was similar to that described by Edney (1951). The animals were weighed immediately before and after exposure, and the difference in weight was taken as a measure of the water lost by evaporation. None of the animals defaecated during exposure. The temperatures used ranged from 10 to 60° C. at 10° intervals. There were four groups of spiders, each consisting of twelve animals; either dead or living and either with or without the lung-books blocked.

The surface area of each spider was established by substitution in the formula $S = kW^{\frac{2}{3}}$, the constant k having been determined by camera lucida drawings of the flattened integuments of three spiders of known weight (k for *Lycosa amentata* = 12·3). Evaporation was then expressed in terms of mg./cm².*/hr.*, and the results are shown in Table 1 and Fig. 2. It should be understood that in

Table 1. *The rate of evaporation of water, in mg./cm²./hr., from* Lycosa amentata *into dry air moving at* ca. 5·0 *cm./sec. Each entry is the mean of twelve determinations, followed by the standard deviation. The minimum measurable rate is approximately* 0·4, *and all means below* 0·6 *are entered as* < 0·6

Temp. ° C.	Alive		Dead	
	Lung-books blocked	Lung-books free	Lung-books blocked	Lung-books free
10	< 0·6	< 0·6	< 0·6	< 0·6
20	< 0·6	0·6 (0·1)	< 0·6	0·6 (0·4)
30	0·6 (0·4)	0·9 (0·5)	0·6 (0·3)	1·6 (0·7)
40	1·25 (0·6)	1·5 (0·5)	1·35 (0·4)	2·1 (0·8)
50	7·6 (1·0)	9·3 (2·2)	10·5 (2·8)	12·4 (1·7)
60	18·7 (3·8)	20·2 (3·0)	20·6 (2·5)	24·6 (3·0)

arriving at the figures which refer to unblocked spiders, no allowance has been made for the surface area of the lung leaflets. The extent to which these are freely evaporating surfaces is unknown, and in any case, a clearer picture of evaporation through the spiracles can be obtained if the results with and without occlusion are expressed in the same terms, i.e. per unit area of *integument*.

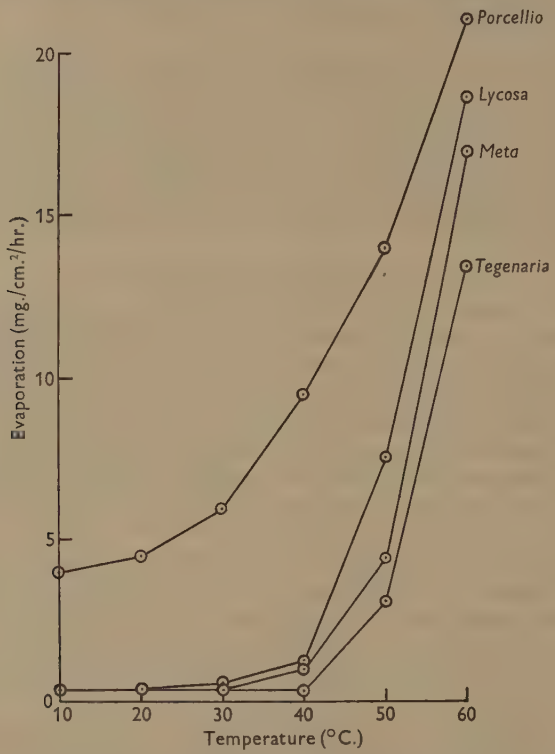

Fig. 2. The rate of evaporation from spiders exposed for 15 min. to dry air at various temperatures. Data for the woodlouse, *Porcellio*, from Edney (1951).

The weight of an individual *Lycosa* is of the order of 20 mg., and the balance used could be read to the nearest 0·1 mg. With these limits, a simple calculation shows that the lowest measurable rate of evaporation is of the order of 0·4 mg./cm².hr., and any value between 0·2 and 0·6 will be read as 0·4. In expressing the results, therefore, means up to 0·6 are not distinguished, but are entered as $<0{\cdot}6$ in the table, and are represented by the value 0·4 in the graph. For this reason little can be said with confidence about the results at 30° C. and below, except that the absolute amounts of water evaporated are very small. At higher temperatures, the differences between living and dead animals, or between those with the spiracles

blocked and free, are not very great; yet it is perhaps significant that wherever differences are observable they are in the same direction, and for both living and dead spiders, the rate of evaporation from intact animals is greater than that from spiders with the lung-books blocked. Furthermore, at all temperatures, the figures referring to dead spiders are higher than the corresponding figures for living ones, whether blocked or free spiders are compared.

After 15 min. exposure at 50° C. the spiders in the 'living' groups died, and they were therefore dead when exposed a few minutes later to 60° C. The fact that these spiders still show a lower rate of evaporation than the corresponding animals in the 'dead' group, which had been killed before the experiment with KCN, is discussed below (p. 580).

The most significant feature of these results from the present point of view is that whereas temperature has little effect upon the rate of evaporation up to 40° C., above this, evaporation increases rapidly, and the shape of the whole curve resembles that obtained with insects.

Six fresh spiders were exposed at intervals of 2° C. from 40 to 50° C., and the evaporation rates shown below in mg./cm.²/hr., indicate a critical temperature at the bottom of this range, since the beginning of the steep rise is already evident at 42° C.:

Temp. °C. ...	40	42	44	46	48	50
Mean	1·3	4·7	5·4	6·5	7·7	10·6
Standard deviation	0·3	1·0	1·2	0·3	0·8	0·8

The spiders used for obtaining this information differed from those to which Table 1 relates in not having been exposed at 10, 20 and 30° C. before exposure at 40° C. The absolute values obtained are not therefore strictly comparable with those shown in that Table, but the position of the break is nevertheless sufficiently clear.

(*b*) *Comparative measurements of the effect of temperature on evaporation from other species*

Comparative measurements of evaporation from the integuments of a number of different species of spider were made in the following conditions: the lung-book spiracles were blocked, the same specimens were used at all temperatures, they were exposed for 15 min. at each temperature, and only female spiders were used (since the males of some species are much smaller and usually rare). The results again expressed as mg./cm².hr., are shown in Table 2 and graphed in Fig. 2 together with the comparable figures for *Lycosa* obtained previously.

Although some of the species used in these measurements are larger than *Lycosa*, so that the minimum measurable rate of evaporation is somewhat lower, it is not considered advisable to attach significance to differences between readings lower than 0·6 mg./cm².hr., and these are again inserted in Table 2 as $< 0{\cdot}6$. In Fig. 2, the value of 0·4 is again used to represent such results graphically.

For purposes of comparison, Fig. 2 also contains a curve showing evaporation from the woodlouse *Porcellio scaber* (data from Edney, 1951), and it is clear that the

curves for all species of spider resemble one another and differ from the *Porcellio* curve in showing the characteristic break at a critical temperature below which evaporation is severely restricted.

Table 2. *The rate of evaporation of water, in mg./cm².hr., into dry air moving at* ca. 5·0 *cm./sec., from spiders exposed for* 15 *min. Each entry is a mean followed by the standard deviation. The minimum measurable rate is approximately* 0·4, *and all means below* 0·6 *are entered as* <0·6

	Lycosa amentata	*Tegenaria derhami*	*Meta segmentata*	*Zilla atrica**	*Zilla x-notata**
No. of spiders used ...	12	14	12	6	6
Temp. ° C.					
10	<0·6	<0·6	<0·6	<0·6	<0·6
20	<0·6	<0·6	<0·6	<0·6	<0·6
30	0·6 (0·4)	<0·6	<0·6	<0·6	<0·6
40	1·25 (0·6)	<0·6	1·0 (0·6)	4·1 (1·2)	1·4 (0·4)
50	7·6 (1·6)	3·2 (1·4)	4·4 (0·7)	13·9 (3·3)	3·0 (0·7)
55	—	—	—	18·9 (6·6)	6·4 (1·6)
60	18·7 (3·8)	13·4 (3·3)	16·9 (2·4)	—	—

* In these two species fresh spiders were used at each temperature (see text).

Evaporation was also measured from *Zilla atrica* and *Z. x-notata*, but since these spiders did not survive repeated exposures so well as other species, fresh spiders were used at each temperature, and the results are therefore not strictly comparable with the rest, for a fresh spider exposed at a high temperature may be expected to lose more water than one which has previously been exposed to a number of lower temperatures. The method does not, of course, affect the determination of critical temperatures. The results for these two species are also given in Table 2.

THE EFFECT OF ABRASION UPON EVAPORATION

In view of the similarity in shape between these curves and those obtained with insects, the presence of a wax layer in the cuticle of spiders may be suspected. In *Rhodnius*, abrasion of the cuticle with an inert dust such as Neosyl apparently removes the wax layer and permits greatly increased evaporation. A similar experiment was carried out with *Lycosa*. Spiders allowed to move in the presence of Neosyl, dusted on them and on the floor of the container in which they were kept, were dead after 24 hr., but they survived well up to 12 hr. After 12 hr. treatment, then, the spiracles were blocked, and evaporation was measured from the integument. Six spiders were used at each of the three temperatures, 20, 30 and 40° C. The results expressed as mg./cm².hr., together with comparable figures for normal spiders, were as follows (means and standard deviations):

	20° C.	30° C.	40° C.
After exposure to Neosyl	2·3 (0·5)	3·6 (1·0)	7·0 (1·7)
Normal spiders	<0·6	0·6 (0·4)	1·25 (0·6)

The increase in rate of evaporation at each temperature after exposure to Neosyl is approximately sixfold, and leaves no doubt that a water-proofing layer has been damaged.

DISCUSSION

Information is now available about the effect of temperature and humidity upon evaporation through the integument of most important groups of terrestrial arthropods. As a result of the work of Ramsay (1935), Wigglesworth (1945) and Beament (1945) the cuticle of many insects has been shown to possess a discrete layer of wax-like material, at or near the surface (or possibly permeating the epicuticle in some species (Kramer & Wigglesworth, 1950)) which limits permeability to water at low temperatures, but which undergoes a physical change, involving loss of orientation of the surface molecules, at a critical temperature, above which evaporation is very much greater.

In the ticks, Lees (1947) has shown that essentially the same mechanism is at work. In both insects and ticks the possession of a higher critical temperature usually means a lesser permeability at temperatures in the biological range, and it is possible roughly to correlate the critical temperature with the kind of biological niche inhabited; the Argasidea, for instance, show critical temperatures which are higher than those in the Ixodidae, and members of the former family inhabit drier, warmer situations than the latter.

As regards the myriapods, Edney (1949) has shown that in the diplopod *Glomeris* there is no sharp break in the temperature-evaporation curve, and that evaporation is approximately proportional to saturation deficit. Cloudesley-Thompson (1950) obtained similar results with another diplopod, *Paradesmus*, and the conclusion is drawn that these animals do not possess a discrete wax layer, but owe to phenolic tanning the modicum of impermeability which is shown by the integument.

Amongst the Crustacea, few of which are terrestrial, the woodlice show the same properties as the diplopods so far as evaporation from the integument is concerned (Edney, 1951), and their cuticle is also believed to be without a discrete wax layer. Different species of woodlice differ as regards permeability and this may perhaps be accounted for by different degrees of tanning (though such a process has not been demonstrated).

Friedel (1928) has investigated the humidity relations of the chilopod *Scutigerella*, but he did not find the effect of temperature upon evaporation, and no information of this nature seeems to be available as regards any member of this group.

So far as arachnids are concerned, the present work on spiders has shown that they resemble ticks and insects in showing a critical temperature, and in showing a greater permeability if the superficial layers of the cuticle are abraded. There seems little doubt, therefore, that they too have a discrete wax layer, and that this has contributed to their success in occupying the drier terrestrial habitats.

It has been shown experimentally that the critical temperature for *Lycosa* is approximately 40° C. Examination of the curves for *Tegenaria* and *Meta* suggest that their critical temperatures are higher. *Tegenaria* certainly shows less than the minimum measurable rate of evaporation at 40° C., and at all higher temperatures

both species show lower rates of evaporation than *Lycosa*. In *Zilla atrica* evaporation has already begun to rise steeply at 40° C.: the critical temperature must be lower than in any other species studied, and the figures suggest 34–36° C. *Zilla x-notata* shows a less well defined critical temperature than most: it is certainly higher than that of *Z. atrica*, but at higher temperatures the rate of evaporation is lower than that of any other species studied.

Such differences as have emerged may well be correlated with habitat, but it is difficult to make such correlation with any confidence, for little is known about the climatic conditions in habitats occupied by spiders. Of the three species other than the *Zillas*, *Tegenaria* shows the highest critical temperature, *Meta* comes next, and *Lycosa* shows the lowest. If the assumption is made that a higher critical temperature denotes a lower rate of evaporation within the biological range (as it does with insects and ticks), then as regards the spiders studied in the present work, it can at least be said that there is some correlation between habitat and resistance to desiccation, for *Tegenaria* is a domestic species, living in sheds, cellars, attics and the like—habitats which often appear to be rather dry. *Lycosa* on the other hand lives in holes in the ground, and is therefore probably in a moister environment. *Meta* occupies a variety of habitats, and shows an intermediate critical temperature.

Of the two *Zillas* investigated, *atrica* shows a lower critical temperature and higher evaporation rate than *x-notata*, and *atrica* occurs on bushes and hedgerows while *x-notata* is found in buildings in the corners of windows and doorways. It seems likely that temperatures will rise higher, and humidities fall lower, in buildings than on hedgerows, but no precise information is available.

As regards evaporation from the respiratory surfaces, the present results show that, like insects, spiders may lose considerable quantities of water in this way. The spiracles leading to the lung-books are provided with closing mechanisms, but if these are kept open artificially by CO_2 (and presumably in nature as the result of activity) evaporation increases by nearly 50% of the normal figure. This is not, in fact, such a large increase as that shown by insects in similar experiments, where evaporation may be more than doubled (Mellanby, 1934).

There is, however, one respect in which spiders appear to behave differently from insects: evaporation is slightly more rapid from dead than from living animals. This effect is observed whether the spiracles are blocked or normally open (see p. 574, and Table 1). In insects, provided the spiracles are sealed, there is no difference in the rate of evaporation from living and dead animals. It seems possible that the explanation may lie in a secretory activity of the epidermal cells. Lees (1947) has shown that unfed ticks absorb water through the cuticle when exposed to relative humidities higher than 90% or so, and that the rate of loss at lower humidities than this is much less rapid if the animals are alive than if they are dead. If spiders possess a similar mechanism, then smaller loss from living specimens would be accounted for by the restraining effect of the secretory activity of the cells concerned. No direct evidence that this is the correct explanation was obtained in the present work, but if the further assumption is made that this secretory activity does not cease immediately upon

death (in the sense of loss of response to mechanical stimuli) but continues for a few minutes at least, then an explanation is available for the observation recorded in Table 1 that at 60° C., although all spiders were dead when exposed, those which had been recently alive continued to lose water less rapidly than those which had been killed by KCN before the beginning of the series of exposures.

SUMMARY

1. The purpose of the present work was to investigate and compare the water-retaining properties of the integument in a number of species of spider. Subsidiary investigations concerned the anatomy and function of the 'tracheae' as respiratory organs, and the significance of these organs and of the lung-books in total water loss.

2. The anatomy of the tracheae was investigated by means of the cobalt-sulphide injection technique. In *Lycosa amentata* they consist of four unbranched tubes, and their surface area is approximately one-thirtieth of that of the lung-book leaflets. Injection of reduced indigo blue demonstrates that O_2 enters via these tracheae, but the amount is too small to be measured by a standard Warburg manometer, and is insufficient to keep the animal alive if the lung-books are blocked. At 30° C. intact spiders absorb approximately 0·6 μl./mg./hr.

3. If the lung-book spiracles are kept open by exposing living spiders to 10% CO_2 in air, evaporation increases by nearly 50% (from 16 to 23% of body weight in 24 hr.). There is no significant increase if dead spiders are exposed, possibly because the spiracles do not open.

4. The rate of evaporation into dry air moving at *ca.* 5·0 cm./sec. was measured from dead and living *Lycosa* with the spiracles either blocked or free. The spiders were exposed for 15 min. at 10° C. intervals from 10 to 60° C. Up to 30° C. the rates in mg./cm².hr. were low, never more than 1·6 (dead spiders with free spiracles) and usually $<0{\cdot}6$. The rate increases rapidly above 40° C., and at higher temperatures, although differences are small, evaporation is always greater from intact than from blocked spiders and greater from dead than from living spiders. Animals exposed at 2° C. intervals from 40 to 50° C. show the beginning of the steep rise at 42° C.—the critical temperature is therefore in this region.

5. Comparable measurements of evaporation from the integument only were made on the following species: *Meta segmentata*, *Tegenaria derhami*, *Zilla atrica* and *Z. x-notata*. Lung-book spiracles were blocked, only females were used, and the same individuals were exposed at each temperature except for the *Zilla* spp. As in *Lycosa*, the rate of evaporation from all these spiders increases abruptly at a critical temperature, and the shape of the curves is similar to that found in insects.

6. The species stand in the following order as regards critical temperatures (lowest to highest): *Zilla atrica*, *Lycosa*, *Meta*, *Tegenaria*. *Zilla x-notata* shows a less well-defined critical temperature, and a lower rate of evaporation than any other species at higher temperatures.

7. Abrasion with an inert dust produces an approximately sixfold increase in the rate of evaporation from *Lycosa*.

8. The above results are compared with similar measurements in other arthropods. Spiders resemble insects and ticks, and differ from isopods and myriapods, so far as the effect of temperature upon evaporation is concerned, and it is suggested that a discrete wax layer is probably present in the spider cuticle.

9. The suggestion that evaporation is resisted by active secretion of the epidermal cells (as in ticks) is put forward to account for somewhat greater rates of evaporation from dead than from living spiders in similar conditions.

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REFERENCES

Beament, J. W. L. (1945). The cuticular lipoids of insects. *J. Exp. Biol.* **21**, 115–31.

Cloudesley-Thompson, J. L. (1950). The water relations and cuticle of *Paradesmus gracilis* (Diplopoda, Strongylidae). *Quart. J. Micr. Sci.* **91**, 453–64.

Edney, E. B. (1949). Evaporation of water from woodlice. *Nature, Lond.*, **164**, 321.

Edney, E. B. (1951). The evaporation of water from woodlice and the millipede, *Glomeris*. *J. Exp. Biol.* **28**, 91–115.

Friedel, H. (1928). Ökologische und physiologische Untersuchungen an *Scutigerella immaculata* (Newp.). *Z. Morph. Okol. Tiere*, **10**, 738–97.

Hazelhoff, E. H. (1926*a*). On a new form of breathing regulation (regulation of diffusion) in insects and arachnids. *Proc. Acad. Sci. Amst.* **29**, 493–6.

Hazelhoff, E. H. (1926*b*). *Regeling der Ademhaling bij Insecten en Spinnen*. Utrecht.

Kastner, A. (1924). Die vergleichende anatomische Bedeutung der Interpulmonarfalte der Araneae. *Zool. Anz.* **58**, 97–102.

Kastner, A. (1929). Bau und Funktion der facher Tracheen einiger Spinne. *Z. Morph. Okol. Tiere*, **13**, 463–558.

Kramer, S. & Wigglesworth, V. B. (1950). The outer layers of the cuticle in the cockroach *Periplaneta americana* and the function of the oenocytes. *Quart. J. Micr. Sci.* **91**, 63–72.

Lamy, E. (1902). Recherches anatomiques sur les trachées des araignées. *Ann. Sci. Nat. Zool.* B, **8**, 149–280.

Lees, A. D. (1947). Transpiration and the structure of the epicuticle in ticks. *J. Exp. Biol.* **23**, 379–410.

Mellanby, K. (1934). The site of loss of water from insects. *Proc. Roy. Soc.* B, **116**, 139–49.

Palmgren, P. (1939). Ökologische und physiologische Untersuchungen über die Spinne *Dolomedes fimbriatus* (Cl.). *Acta. zool. Fenn.* **24**, 1–42.

Ramsay, J. A. (1935). The evaporation of water from the cockroach. *J. Exp. Biol.* **12**, 373–83.

Remy, P. (1925). *Contribution à l'étude de l'appareil respiratoire et de la respiration chez quelques invertébrés*. Nancy.

Wigglesworth, V. B. (1945). Transpiration through the cuticle of insects. *J. Exp. Biol.* **21**, 97–114.

Wigglesworth, V. B. (1950). A new method for injecting the tracheae and tracheoles of insects. *Quart. J. Micr. Sci.* **91**, 217–24.

ANAL AND ORAL INTAKE OF WATER BY CRUSTACEA

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In small and transparent Crustacea water can be seen with a microscope to be pumped through the anus into the intestine. The pumping is done by antiperistaltic contractions of rectal muscles, the movements being repeated rhythmically at intervals of one to a few seconds. They may continue almost without interruption or may occur for short periods with intervals of rest. In some genera the entrance of water by successive gulps is quite clear; this is the case, for example, in *Triops*, *Sida*, *Diaphanosoma*, *Leptodora*, *Mysis*, *Sphaeroma*, *Ligia*, *Corophium*. In other genera, such as *Artemia*, *Chirocephalus*, *Limnadia* and *Bythotrephes*, it is not obvious that water enters, although waves of contraction are seen to pass inwards over the rectal musculature. In *Daphnia* the gulps cannot be seen when the antiperistalsis is slow, but they are clearly visible when it is rapid.

PREVIOUS WORK

Rectal swallowing of water in Crustacea seems first to have been observed by Lereboullet (1850). He saw it in *Limnadia*, *Daphnia* and newly hatched crayfishes. In the latter he watched carmine particles taken into the rectum and expelled again. Lereboullet interpreted the phenomenon as one of anal respiration, without giving any reasons for this opinion. Presumably it seemed to him that the entrance and exit of water into and out of the gut of an aquatic animal could have no other function.

Weismann (1874), in his thorough and beautifully illustrated study of *Leptodora*, described the swallowing of water through the anus, the rectal dilator muscles acting as a suction pump and the circular muscles as a force pump. Carmine particles were taken in. He, too, stated that the function of the intake of water is respiratory. He pointed out, moreover, that water is also swallowed rhythmically through the mouth, and he considered the whole gut to be respiratory.

Siedentop (1930) made experiments with *Leptodora* to test Weismann's view. If the anal intake of water has a respiratory function, its rate might be expected to increase in water deficient in dissolved oxygen, as has since been found for the respiratory organs of various Crustacea (Fox & Johnson, 1934; Johnson, 1936; Peters, 1938; Walshe-Maetz, 1952). In Siedentop's experiments a low oxygen content of the water doubled the rate of swallowing movements.

ANAL INTAKE OF WATER

I have confirmed and extended Weismann's observations on *Leptodora kindti* (Focke) by a study of forty-nine individuals at 20–23° C. (Fox, 1952). All animals, although examined as quickly as possible after capture, had empty stomachs, the

walls of which showed continuous and vigorous antiperistalsis, with a mean rate of 14 contractions per minute. The anal intake of water by rhythmic antiperistaltic movements of the rectum was clearly seen. In some individuals it went on for long periods, with few interruptions, while in others it occurred only in occasional bursts of rhythmic rectal contractions. When more or less continuous, the swallowing movements were interrupted at intervals by a pseudo-defaecation of water, which got rid of at least some of the water that was pumped in. In a typical case the mean rate of rectal swallowing was 16 gulps per minute, and four successive pseudo-defaecations occurred after 25, 38, 27 and 34 gulps. Usually the swallowing movements stopped for a few moments after each defaecation, in other cases their rate doubled just before defaecation and slowed just afterwards.

Lereboullet (1850) had found that a carmine suspension is swallowed by the rectum of young crayfishes, and Weismann (1874) repeated this with *Leptodora*. I have been able to show that various other Crustacea can swallow suspensions through the anus. For example, a young individual of *Lepidurus* sp., 7 mm. long (hatched from New Zealand mud), was watched with a low-power microscope beneath a supported cover-slip. After defaecation it often happened in these circumstances that the faeces remained close to the anus; portions of the faeces were then frequently taken back into the intestine. Another way of showing the phenomenon is to put animals into a suspension of *Chlorella*. These green algal cells were seen to be taken into the hindgut of metanauplii of *Triops cancriformis* (Bosc). In the cladoceran *Sida crystallina* (O. F. Müll.) with an empty intestine the cells of *Chlorella* were seen to be ingested through the anus and later to be re-expelled. The rate of swallowing was, in three individuals, 43, 46 and 47 gulps per minute at 18° C. In one individual the numbers of gulps between successive expulsions of *Chlorella* were 11, 13, 17, 14, 17, 17 and 19, in another 45, 22, 26, 21, 15 and 56. These expulsions of rectally swallowed *Chlorella* really correspond to normal defaecations, for in another individual, which had food in the gut and had been given no *Chlorella*, the numbers of rectal gulps of water betweeen successive expulsions of faeces were of the same order of magnitude, namely, 37, 25, 30 and 36. In *Hemimysis lamornae* (Couch), too, the cells of *Chlorella*, suspended in the sea water, were seen to be taken into the rectum, and, in individuals with an intestine devoid of faeces, the algal cells were quickly passed forwards as far as the front of the abdomen by the antiperistaltic contractions of the intestine wall.

I have observed rhythmic swallowing of water through the anus in the majority of small and transparent Crustacea examined, including the following, in addition to species already mentioned: *Branchipus stagnalis* (L.), *Chirocephalus diaphanus* Prévost, *Artemia salina* (L.) in sea water, *Limnadia lenticularis* (L.), *Leptestheria mayeti* (Simon), *Diaphanosoma brachyurum* (Liéven), *Penilia avirostris* Dana, *Daphnia* spp., *Moina brachiata* (Jurine), *Bythotrephes longimanus* Leydig, *Calanus gracilis* Dana, *Temora stilifera* Dana, *Asellus aquaticus* (L.), *Sphaeroma* sp., *Ligia mediterranea* F., *Corophium volutator* (Pallas), the isopod *Astacilla deshayesi* Luc., the amphipods *Phronima atlantica* Guér. and *Phtisica marina* Slabbor, *Euphausia* sp., various prawns named below, and zoaea and megalopa larvae. I have not, however,

been able to see the phenomenon in *Evadne nordmanni* Lov., *E. spinifera* P. E. Müll., *Podon leuckarti* Sars, *Heterocope saliens* Lilljb., *Diaptomus* sp., *Cyclops strenuus* Fischer, *Argulus foliaceus* (L.), or *Gammarus* spp. A varying number of each species was studied.

In the smallest Crustacea the rectal swallowing of water is continuous. This is so both in small adults and the young of big forms. In larger animals there are pauses between bursts of swallowing movements. The following are examples. In metanauplii of *Triops* and in half-grown animals of this genus, and again in *Sida*, the water-swallowing is continuous, interrupted only momentarily by the frequent defaecations. In *Hemimysis* there are bursts of from two to three up to a couple of dozen successive rectal antiperistaltic contractions, with intervening still periods of irregular length without any pumping. In big prawns the water intake seldom occurs except before and just after defaecation. We have here a series of animals of increasing size: the larvae of *Triops* are almost microscopic, the length of *Sida* is 3 mm., of half-grown *Triops* 7 mm., of *Hemimysis* 10 mm., whilst prawns are the largest Crustacea transparent enough for study. Again, within one small species of prawn, *Hippolyte prideauxiana* Leach, rectal swallowing of water is continuous, or nearly so, in very small animals, whether the gut is full or empty of food. In bigger members of this species rectal swallowing is rare with an empty gut, and with food in the gut it only occurs at the time of defaecation. The critical length of animal for continuous rectal water intake at 22° C. was found to be 5 mm. At 4 mm. and below the swallowing is continuous, at 6 mm. occasional, and above this length, up to 10 mm. (the length of the biggest individuals of this species transparent enough for study), it is rare except at the time of defaecation. Eighteen individuals were studied.

The rate of rectal swallowing of water is moderately constant in the case of continuous intake by small individuals, but it is very variable in bigger animals and may change in a single individual from 20 to 120 gulps per minute.

DEFAECATION IN PRAWNS

From what has been written above, it is obvious that there is some relation between rectal swallowing of water and defaecation. This becomes clearer from a further study of prawns. *Periclimenes scripta* (Risso), a Mediterranean species, is particularly suitable owing to its great transparency. The length of individuals studied was about 2·0–2·5 cm. At most times the rectal wall is seen to be quite still. Then rectal antiperistalsis with resulting water intake may begin, at first slowly, then more and more rapidly, with finally about one contraction per second, until, after some 20–40 pulsations, defaecation occurs. This series of events may be repeated 4 times in 10 min. at 22° C. Each time, after defaecation has occurred, up to five further quick rectal inward pumping movements follow, as if to replace with water the volume of the extruded faeces, and then all is still.

In this species each gulp of water pumped inwards by the rectal swallowing movement can be seen to be passed right up to the front end of the abdomen by a corresponding antiperistaltic contraction of the intestinal wall. When defaecation

occurs, without there being any arrest of the rectal and intestinal antiperistalsis, a cylinder of gelatinous faeces which was lying at the front end of the intestine quickly descends the whole length of the abdomen and is extruded. What causes the descent of faeces cannot be seen owing to the opacity of the thorax; it could be brought about by a contraction of the gut wall anterior to the piece of faeces. The descent of faeces is rapid; in one case it took 8 sec. for a piece 1 mm. in length to move 12 mm. from the anterior end of the intestine to the anus. The cylinder of faeces passes down the intestine with some water just before it and just behind it, thus elongating the swelling in the otherwise narrow intestinal tube.

It is clear that the process of pumping water in through the anus is really a natural enema. Each time that rectal inward pumping of water starts it can be prophesied with certainty that defaecation will take place. The events described only occur if there is food in the gut; when the gut is empty there is, in these prawns, almost always a complete absence of rectal or intestinal contractions. If such fasting prawns are given food, faeces may be expelled a quarter of an hour afterwards, always with rectal swallowing of water.

In the prawns *Leander adspersus* (Rathke) and *L. squilla* (L.), just as in *Periclimenes*, intestine and rectum are usually still, until defaecation is preceded and accompanied by the rectal intake of water. In *Leander* each defaecation is followed by a dozen or so swallowing movements, which are quicker than before the event. Sometimes, but not usually, single rectal swallowing movements occur when the intestine is empty of faeces; this may even be repeated rhythmically at long intervals of about 10 sec., each rectal movement being followed at once by a single intestinal antiperistalsis. Occasionally, but rarely, intestinal antiperistalsis was seen without any rectal swallowing. These observations were made at 22° C. on half-grown animals, 2·0–4·5 cm. long, bigger ones being too opaque for observation.

The little prawn *Hippolyte prideauxiana* is somewhat different from *Periclimenes* as regards defaecation, in that the cylinder of faeces, instead of descending the intestine quickly, moves slowly along it and is more than half-way down before the pumping in of water begins. The intestine is widest just in front of the rectum, becoming progressively narrower forwards; at the front of the abdomen the faecal strand fills the intestine, at the hind end it does not nearly fill the lumen and there is plenty of room for water to flow forwards past the faeces. Sometimes, when the strand of faeces has passed partly out of the anus, the rectal pumping inward of water stops; then the exit of the faeces stops too, until rectal swallowing has begun again, when defaecation recommences. This may occur several times before the faecal strand has passed the anus. In watching this it looks as if the water which is pumped into the intestine makes the faeces come out. With *Hippolyte varians* Leach, too, this is the impression gained. In *Hippolyte* there is, once again, a quick short burst of vigorous rectal and intestinal antiperistalsis immediately after defaecation. In this genus intestinal antiperistalsis usually accompanies rectal swallowing, but the contractions in the rectum and intestine are not always in step with one another as in *Periclimenes* and *Leander*, although the intestinal movements are invariably accelerated when rectal gulps become more rapid (they may reach

a speed of 2 per second). Rectal and intestinal antiperistalsis can, however, each occur without the other, and there is often antiperistalsis at the front end of the intestine with none behind. The wave-length of the intestinal movement decreases progressively as the wave passes forwards.

In the fresh-water prawn *Atyaephyra desmaresti* (Millet), of 2–3 cm. in length, rectal and intestinal antiperistaltic waves were often seen with an empty intestine. Six animals were studied at intervals during 4 successive days. On 42 occasions rectal swallowing of water was seen, while on 19 occasions there was none. Out of the 42 occasions the intestine was empty on 18. This was unlike the behaviour of the marine prawns dealt with above, in which rectal pumping rarely occurred without defaecation. As usual, however, the pumping in *Atyaephyra* became quicker before defaecation and remained quick just after it, when the gulps of water were bigger.

Yet the anal intake of water with an empty intestine seems not to be a speciality of fresh-water prawns, for in the Italian fresh-water race of *Palaemonetes varians* (Leach), seven out of eight animals studied showed no rectal antiperistalsis without defaecation.* The English race of *P. varians*, observed both in sea water and brackish water, again showed no anal intake of water without defaecation; at nearly all times rectum and intestine were still.

In prawns, as already described, after the rectal swallowing of water has started, it becomes more and more rapid until defaecation occurs; it continues for a few moments after this and then it stops. In adult *Artemia salina* (in sea water) rectal swallowing occurs only just before defaecation and there is none after the event, but in younger *Artemia* there is continuous swallowing which only stops for a few moments after defaecation, while in the metanauplii the swallowing does not stop but it is more rapid and vigorous just before defaecation. In metanauplii of *Leptestheria mayeti* the otherwise continuous anal water intake, with a rate of about 40 gulps per minute at 18° C., pauses after each defaecation. Likewise in metanauplii of *Limnadia lenticularis* the swallowing movements, with a rate of 75 to the minute at 23° C., were continuous except for a pause of about 20 sec. after each defaecation, which took place every 1–2 min. It is curious that the rectal swallowing of water should continue for a short time after defaecation in prawns, whereas, on the contrary, it stops after defaecation in phyllopods.

Why is it necessary for Crustacea to have this peculiar mechanism of rectal pumping in of water to bring about defaecation? Why must the circular muscles of the gut wall be stretched hydrostatically for the animal to defaecate? The reason

* One individual *P. varians* was observed to swallow water through the anus when the intestine was empty, behaving in a way which I have not otherwise seen in prawns. There were periodic bursts of rectal swallowing of water; 13 successive bursts were observed (at 23° C.), each burst having an average of 27 gulps with a rate of about 1 gulp per second, followed by an average pause of 28 sec. Intestinal antiperistalsis accompanied each burst of rectal pumping, otherwise there was none. At the end of each burst of rectal swallowing, a sausage of water descended the intestine as if it had been a piece of faeces, then one or two more gulps followed, after which came the pause. This was a peculiar case of rhythmic pseudo-defaecation. It corresponds to the normal occurrence in small Crustacea which show continuous anal swallowing of water proceeding unabated when the gut is empty, with momentary interruptions for a pseudo-defaecation of water. This was described above in *Leptodora*. and in *Sida* which had been made to swallow *Chlorella* through the anus.

may be, in part at least, that Crustacea have an exoskeleton and therefore no muscular body-wall. We ourselves assist defaecation by the contraction of abdominal body-wall muscles, and so do birds, but a crustacean has no such muscles.

FUNCTIONS OF THE ANAL INTAKE OF WATER

The events in prawns make it seem certain that in these animals the rhythmic intake of water through the anus is of the nature of a natural enema. The same strong impression comes from observing other Crustacea. Defaecation was watched, for example, in a zoaea larva from the Mediterranean plankton. It is unusual to see defaecation in plankton larvae because their gut is nearly always empty by the time that they are examined in the laboratory. As the faecal strand slowly crept down the intestine, the rectal swallowing of water and intestinal antiperistalsis became quicker and more continuous. When defaecation took place there was a violent burst of quick rectal and intestinal antiperistalsis, and after defaecation this continued for a moment and then stopped. In metanauplii of the conchostracan *Eoleptestheria ticinensis* (Criv.) rectal swallowing stopped after each defaecation, then after a pause it started feebly, becoming increasingly vigorous, with bigger gulps, until the next defaecation. In metanauplii of *Limnadia lenticularis* no actual gulps could be seen in the rectal antiperistalsis, but water is nevertheless swallowed and it visibly distends the posterior portion of the midgut more and more until defaecation occurs.

The spells of anal water intake in small Crustacea become progressively more frequent as the size of animal decreases; the pauses between bursts of swallowing become shorter, although the rate of the rhythmic rectal contractions does not increase. In the smallest animals there are no pauses and the rhythmic swallowing of water is continuous. This shortening and final disappearance of pauses goes together with more and more frequent defaecations as the size of animals diminishes. Doubtless the greater frequency of defaecation in small crustaceans corresponds to their higher rate of metabolism (Hotovy, 1938; Weymouth, Crismon, Hall, Balding & Field, 1944; Zeuthen, 1947) and consequent necessity of eating more. In the small animals, with continuous rectal swallowing of water and frequent defaecation, there is no reason to think that the function of the water intake as an enema is not the same as in prawns.

Water may, however, also be taken in through the anus when there is no food in the gut, or at least in the hindgut. This, as we have seen, occurs regularly in small animals and occasionally in large ones. Since the water acts as enema, why should it be pumped inwards in the absence of faeces? It may be that in nature there are nearly always faeces in the intestine. Animals are examined in a laboratory some time after capture, when no doubt food is no longer available. The rectal inward pumping of water, evolved to deal with an intestine normally containing faeces, may well continue automatically with an abnormally empty gut. But the continuous anal drinking by small Crustacea, when the gut is empty, may indicate that the swallowed water has a second independent function, additional to that of an enema. If so, what could this function be?

Lereboullet and Weismann thought that the absorption of water through the anus has a respiratory function.* Siedentop (1930) considered that he had proved the truth of this view by showing that in *Leptodora* paucity of dissolved oxygen in the outside water increases the rate of rectal inward pumping. But I cannot agree with the opinion that the anal intake of water is respiratory. My reasons are the following.

First, I have tried to confirm Siedentop's experiments, but have failed to do so. Using, as he did, *Leptodora kindti*, I could get no increased rate of rectal anti-peristalsis in any of six animals by putting them into water through which nitrogen had been bubbled to give various deficits in dissolved oxygen. Nor, in four other animals, could I increase the rate with carbon dioxide. I had similar negative results on the rate or volume of the rectal swallowing of water by *Bythotrephes longimanus*, using nitrogen (eight animals) and carbon dioxide (seven animals). Moreover, *Daphnia obtusa* Kurz did not increase its rate or volume of anal swallowing when enclosed in a drop of water with no air surface, under which conditions the oxygen was progressively diminished and carbon dioxide increased by the metabolism of the animals themselves.

Secondly, the quantity of water taken in through the anus has the appearance of being insignificant compared with the amount flowing continuously past the gills in animals where these exist, as in Decapoda, Isopoda and perhaps Branchiopoda. This was particularly striking in small and therefore translucent isopods, where the rectal inward pumping of water and the gill movements could be observed simultaneously. In a typical case a young individual of *Sphaeroma* sp., 2·5 mm. long, swallowed water through the anus continuously and slowly over a period of half an hour with one gulp about every 35 sec. while a continuous rapid stream of water passed over the vibrating pleopods. The size of the rectal gulp of water was very small indeed compared with the area of the pleopods.

Thus the anal intake of water does not seem to have a special respiratory function, although, of course, the oxygen dissolved in the relatively small quantity of water swallowed must be used in respiration by gut epithelial cells. It seems probable, however, that the main oxygen supply of these cells must be from the respiratory organs through the blood stream.

Two other possible functions of anal water intake have suggested themselves. One of these is salt absorption. Fresh-water animals necessarily have body fluids richer in dissolved salts than the water in which they live. They are therefore obliged to collect ions from the water and to continue to do so, since they cannot avoid losing some ions. If salt collection were the function of the anal intake of water, the swallowing should be more developed in fresh-water than in marine

* Jančařík (1949) has observed that *Daphnia* and *Moina* occasionally suck air into the intestine through the rectum and that the bubble is resorbed through the gut wall. He considers this to be evidence in support of intestinal respiration. But if a bubble of air enters the gut it must inevitably decrease in size as its oxygen diffuses into the tissues, where, owing to tissue respiration, the oxygen pressure is lower than in the bubble. Moreover, the swallowing of air must be very exceptional as it has never been observed in this laboratory where living *Daphnia* has been studied continuously through the last decade.

animals. But this is not so. In the marine cladoceran *Penilia avirostris* there is anal water intake just as there is in the nearly related fresh-water species *Diaphanosoma brachyurum* and *Sida crystallina*. The marine copepods *Calanus* and *Temora* swallow water through the anus, but the fresh-water genera *Cyclops*, *Diaptomus* and *Heterocope* were not observed to do so. Putting the brackish-water isopod *Corophium volutator* into sea water and into fresh water does not alter its rate or periods of rectal pumping. Further evidence against an osmoregulatory function was supplied by the brine shrimp, *Artemia salina*. Three-day metanauplii were studied, derived from parents reared in sea water of normal, of triple and of half concentrations. In each of the three waters, ten larvae were studied. For each larva three counts were made of the time (to 0·1 sec.) for ten rectal swallowing movements at 17° C. The mean times in seconds with their standard errors were: in sea water $22{\cdot}6 \pm 0{\cdot}3$, in brine $23{\cdot}2 \pm 0{\cdot}7$, in brackish water $22{\cdot}2 \pm 0{\cdot}9$. Clearly there is no significant difference in the rates, nor was there any visible difference in the volumes swallowed. There is thus no evidence for an osmoregulatory function for the anal water intake. Nevertheless, in animals with a relatively impermeable exoskeleton, salt entry into the body must take place at least partly through the gut wall.

Another possible function for the continuous rectal pumping in of water by small Crustacea, even with an empty gut, might be to maintain the turgor of the body, thus keeping the not very rigid exoskeleton stretched. No doubt turgor is largely maintained osmotically; in *Daphnia magna* Straus, for instance, the blood has an osmotic pressure well above that of the outside water (Fritsche, 1917–20). But if turgor is kept up not only osmotically but also hydrostatically by the rectal pumping inwards of water, then the rate of pumping or the volume taken in should vary at different stages of each instar, since the exoskeleton is softest just after the moult. In *Daphnia* an instar lasts, at room temperatures, from 2 to 3 days, and the stage in the instar at any given moment can be told from the stage of development of parthenogenetic embryos in the brood pouch, since eggs are laid in the pouch just after the moult and the young swim away just before the next moult. A thorough study was made of variations in rectal water swallowing movements in relation to stages of the instar of *D. obtusa* but no correlation was found as regards the rate. The volume of water swallowed in *Daphnia* can be seen to increase when the rate of swallowing augments, but no relation was found between volume and instar stage. This hypothesis was therefore abandoned.

It seems, thus, that while the anal intake of water by Crustacea acts as an enema, it does not serve for respiration, osmoregulation or the maintenance of turgor. In prawns, antiperistaltic contractions of the long intestine carry the swallowed water forward to the front of the abdomen and continue to do so until defaecation occurs. In *Periclimenes* the intestinal antiperistalsis nearly always depends strictly on the rectal swallowing: each rectal inward pumping movement initiates an intestinal antiperistaltic wave. In *Leander* the same is usually true, although occasionally the intestinal movement was seen without the rectal. In *Hippolyte* intestinal antiperistalsis generally occurs only with rectal swallowing, although the two are not

always in step; yet the former accelerates when the latter does so. Sometimes in *Hippolyte* intestinal antiperistalsis was seen without rectal antiperistalsis, but it was then less vigorous than usual; often, however, antiperistalsis occurred at the anterior end of the intestine with none at the posterior end. In decapod larvae all states of dependence and independence of intestinal and rectal antiperistalsis were seen. Again, in the marine copepods *Calanus* sp. and *Temora stilifera* each swallowing movement of the rectum was continued forwards as an intestinal antiperistalsis right up to the thorax; the mean rate in *T. stilifera* was 26 per minute at 23° C. In a few Crustacea, as *Triops* and *Gammarus*, intestinal antiperistalsis was not seen, but in *Triops* there are antiperistaltic contractions in the thoracic part of the alimentary canal. Thus rectal swallowing usually causes intestinal antiperistalsis.

The action of the water pumped into the gut by the rectum, both as an enema and as an initiator of intestinal antiperistalsis, seems to be due to the contraction of the circular muscles of the gut wall in response to stretching by hydrostatic pressure. Bayliss & Starling (1899) showed that distention of the colon in a rabbit results in peristalsis, and no doubt this is the mode of action of a human water enema. Similarly water pumped into the crustacean gut through the anus results in muscular contraction causing defaecation, with a reflex relaxation of the rectum, which is normally closed like a sphincter except for the gulps of swallowed water which pass periodically inwards.

The action of the water swallowed through the anus in initiating intestinal antiperistalsis recalls the well-known properties of molluscan heart muscle (Ranson, 1884; Biedermann, 1884; Straub, 1901; Carlson, 1906; Fredericq, 1913; Koch, 1917; Dubuisson, 1930, and others). If a molluscan heart is emptied of blood, it usually stops contracting, or it only contracts feebly. If it is filled again with fluid, contractions start, or increase in strength. The greater the pressure of liquid in the ventricle, the greater is the strength of contraction, and also the faster is the rate of beat. In vertebrate animals, too, the energy of muscular contraction is proportional, within limits, to the length to which muscle fibres are stretched. Starling's 'law of the heart' states that increase in diastolic length of ventricular muscle fibres, distended by additional blood, increases the energy of subsequent systolic contraction. In the prawn's intestine, likewise, hydrostatic pressure due to water pumped inwards by the rectum initiates and maintains rhythmic antiperistalsis of the intestinal wall.

After defaecation in prawns a few more rectal swallowing movements continue, as if to fill with water the space previously occupied by faeces. This may be necessary in order to stretch the gut wall just enough to maintain the tone of its muscles, without starting intestinal antiperistalsis again, which has now stopped and does not recommence until the next burst of rectal water-swallowing, preceding the next defaecation.

ORAL INTAKE OF WATER

Weismann (1874) described the rhythmic swallowing of water through the mouth of *Leptodora*, its pumping inwards by the longitudinal and dilator muscles of the gullet, and its passage down the long 'oesophagus' to the 'stomach', brought about

by peristalsis of the oesophageal wall.* He interpreted the oral, just as the anal, intake of water in *Leptodora* as respiratory. At times, particularly with an empty stomach, he saw that water is moved forwards in the oesophagus, antiperistaltically.

The oral intake of water by *L. kindti* can only be seen in a side view of the animal. It consists of a rhythmic peristaltic swallowing movement in the short gullet, which runs dorsally from the mouth. In most individuals studied I found this drinking to be continuous, rapid and vigorous, the gulps of water being big. The mean rate of swallowing movements in six animals was 102 gulps per minute at 20° C. The water is passed down the long oesophagus into the stomach by a much slower peristalsis, which had, in ten animals, a mean rate of 5 movements per minute. The water can easily be seen entering the stomach because of its slightly different refractive index on arrival. Occasionally an antiperistaltic movement occurred in the oesophagus.

This continuous rhythmic oral intake of water is to be seen in the majority of Crustacea transparent enough to show it. The rhythm is much more constant than that of rectal swallowing. I have found rhythmic oral swallowing of water in the following species: *Triops* sp. (young, 2·5 mm. long) (78)†, *Branchipus stagnalis* (25), *Limnadia lenticularis* (27), *Leptestheria mayeti* (86), *Penilia avirostris* (37), *Diaphanosoma brachyurum* (29), *Daphnia hyalina* Leydig (24), *Moina brachiata* (43), *Bythotrephes longimanus* (95), *Argulus foliaceus* (86), *Gammarus* sp. (30), the mysid *Siriella armata* Claus (100), *Periclimenes scripta* (21), *Hippolyte varians* (33), *Palaemonetes varians* (21), and in decapod larvae. In *Artemia salina* (in sea water), *Argulus foliaceus* and *Palaemonetes varians* the rhythmic swallowing of water through the mouth was continuous in some individuals, intermittent with still pauses in others, and absent in yet others. It could not be seen in *Podon* or *Evadne*, but the gullet is not clearly visible. The rhythmic oral water-swallowing was absent in young *Asellus* 1·25 mm. long (adults are too opaque to examine) and in the copepods *Calanus* sp., *Temora stilifera*, *Diaptomus* sp., *Heterocope saliens* and *Cyclops strenuus*, but was observed, as frequent bursts of rapid peristalsis of the gullet, in one individual of *Eucalanus* sp. A varying number of each species was studied.

In all the above-mentioned forms which show oral drinking—continuous, intermittent, or at least present in some of the individuals studied—anal water intake is also found, with the exception of *Argulus* and *Gammarus*. In three of the forms in which no oral swallowing of water was observed, there was anal intake, namely in *Calanus*, *Temora*, and *Asellus*, but in *Heterocope*, *Podon* and *Evadne* neither oral nor anal water intake was seen. Thus, except for the last-mentioned forms, in all the Crustacea studied water is pumped into the alimentary canal from both ends, or from either end, continuously or intermittently.

A curious detail in the rhythmic oral drinking by *Daphnia* and *Limnadia* is that

* In *Leptodora* the narrow anterior part of the midgut is known as the 'oesophagus' and the posterior wide part as the 'stomach'. In other Crustacea the foregut, here called the 'gullet', is often called the 'oesophagus'.

† The figures in brackets denote the mean number of swallowing movements per minute. The temperatures were between 17 and 23° C., but for simplicity are not given.

each gulp passed down the gullet corresponds to a movement of the jaws. Usually water is swallowed at every second jaw movement, but if for a while the mandibles are grinding quickly, water is swallowed at each third or even fourth movement, whereas if jaws are moving slowly, water is swallowed with every movement. Yet, in spite of this regularity, swallowing is not inseparably linked to jaw movement, for sometimes the jaws stop, but the rhythmic drinking continues uninterrupted.

Greater quantities of water seem to be taken in through the mouth than through the anus: the gulps are bigger. This is the impression gained in spite of the rate of swallowing being often more rapid in anal than in oral water intake. For example, in one individual of *Daphnia hyalina* at 24° C. the rectal swallowing rate was 43 and that of the gullet 23 per minute.

FUNCTIONS OF ORAL WATER INTAKE

If the anal intake of water is not respiratory, there is no reason to think that the oral intake has this function, in spite of what Weismann supposed. In filter-feeding or vortex-feeding Crustacea it might be thought that a continuous rhythmic swallowing of water through the mouth was part of the feeding mechanism. Since the limbs are more or less continuously collecting unicellular algae or detritus suspended in the water, this food must be swallowed continuously. In *Limnadia lenticularis*, however, the limb movements can be temporarily stopped by mechanical shock if the microscope slide on which the animal lies is dropped on the stage of the microscope, yet when this is done the swallowing movements continue undisturbed; thus limb and gullet movements are independent. Again, with decapod larvae the limbs can be held still by a cover-slip of a compressorium without disturbing the regular swallowing movements of the gullet. Moreover, the continuous rhythmic drinking is found in predators, for instance *Leptodora* and *Bythotrephes*, and in scavengers such as prawns, just as much as in plankton feeders.

Thus most Crustacea pump water into the gut both through the mouth and through the anus, or through one or other of the two apertures. We have deduced the function of the rectal swallowing of water, namely to stretch the wall of the gut; rhythmic oral drinking may well have a similar function of distending the gut wall with the result that its muscles act effectively.* But why some copepods require neither oral nor anal drinking is unexplained.

In Cladocera (other than *Leptodora*) the whole midgut, between foregut (gullet) and hindgut (rectum), is often called the 'intestine'. This is clearly different in function as well as origin from the 'intestine' of Decapoda, which is a hindgut passage for faeces from midgut to rectum. In Cladocera the intestine, with the food confined within a peritrophic membrane, serves for digestion of food, absorp-

* In the marine cladocerans *Podon* and *Evadne*, although I failed to see either oral or anal water intake, antiperistaltic contractions of the midgut wall were continuous. There was also a continuous, more rapid, rhythmic contraction by the short, wide anterior gut caeca. In *Evadne nordmanni* at 22° C. the gut wave frequency was once in 40 sec., that of the caeca once in 5 sec. The effect of the caecal contractions would be to keep up a rhythmic pressure on the water in the gut lumen, thus distending the gut walls, and so, presumably, enabling their muscles to contract effectively. It would be interesting to know if the caeca have a more powerful musculature.

tion of digested products and the preparation of faeces. The walls of the cladoceran intestine can be seen to undergo antiperistaltic* contractions which must serve to mix food and enzymes. In *Daphnia*, *Bythotrephes* and other Cladocera there is no relation between the intermittent rhythmic swallowing of water by the rectum and the intestinal antiperistalsis. In *B. longimanus*, for example, intestinal antiperistalsis was seen to be fairly regular with 46 movements per minute at 24° C., while the irregular rectal antiperistalsis varied between 120 movements per minute and no movements at all. In *Daphnia* intestinal antiperistalsis is usually continuous, although in a given individual the waves vary much in amplitude at different times. In the posterior part of the intestine the wave-length is short and the rate of contraction rapid, in the middle part the wave-length is long and the rate is less rapid, while in the anterior part there are no waves. In *D. hyalina* the mean number of antiperistaltic intestinal waves in six individuals was 106 per minute in the posterior region and 45 in the middle region of the intestine at 23° C.

The peritrophic membrane containing the food is, in the anterior part of the intestine of *Daphnia*, separated by some distance from the intestinal wall, the gut lumen here being wider than it is farther back, where the membrane touches the wall. Unless the animal is feeding very actively, that is unless it is swimming in a thick suspension of particles, the anterior third or half of the peritrophic membrane is empty of food. The posterior two-thirds or half of the peritrophic membrane is normally full of food undergoing digestion, the hindmost portion of the food being in the state of faeces ready for defaecation. The short rectum is free of faeces, its lumen being closed except for the periodic pumping in of water from the anus to the intestine. When defaecation takes place, which may occur several times in a minute, a short length of faeces is expelled in an instant through the rectum and anus.

The food within the peritrophic membrane is continuously moved to and fro by the antiperistalsis of the intestinal wall. Posteriorly the rapid, short antiperistaltic waves churn up the food. In the middle region of the intestine each of the slower and more powerful waves moves the food forwards a little way within the peritrophic membrane into its anterior empty region, and then the food at once moves back again after the wave has passed. In moving back again, the hindmost portion of the food which had been forced forwards starts to move first, followed immediately by the front portion. As the food moves forwards again, the anterior intestinal wall can be seen to be extended slightly by the liquid forced forward between peritrophic membrane and wall. As the food returns to its first position, the swollen anterior intestinal wall contracts. This contraction, whether muscular or merely elastic, would help the gut liquid and food to return back where they came from. The movements described must result in mixing the food and digestive enzymes.

* One wonders why these movements are antiperistaltic, not peristaltic. In the Decapoda antiperistalsis of the hindgut intestine clearly serves to move the water, which has been pumped in by the rectum, forwards along the intestine, and past the faeces which might otherwise act as a plug causing the swallowed water merely to swell out the hind portion of the intestine. As it is, the water is moved forward and stretches the gut until defaecation takes place. But in the cladoceran midgut intestine a backward peristaltic movement would presumably have served as well as a forward antiperistaltic movement to churn up the food: perhaps an antiperistaltic movement is inherent in crustacean organization, as seen in the rhythm of movement of phyllopod and other limbs.

The two caeca at the front of the intestine of *Daphnia* normally contain no food; they contract together a fraction of a second after the food within the peritrophic membrane, forced forwards by intestinal antiperistalsis, starts to move back again. The contraction period of the caeca is thus the same as that of the middle intestinal antiperistalsis.* The contraction of the caeca is apparently muscular and this too would assist the food to go back to its original position. Be this as it may, the liquid in the caeca is partly renewed at each contraction and subsequent expansion, and this must assist any digestive or absorptive function, as yet unknown, which the caeca may have.

This account of the movements of food in the intestine of *Daphnia* shows the important part played by the rhythmic movements of the intestine wall. A function of the oral as well as anal water intake may be to stretch the muscles of the gut wall so that they may contract rhythmically and effectively. In other Crustacea oral water intake would have an analogous function.

The rhythmic intake of water through the mouth may, in addition, have another function connected with the movement of food in the gut. In Cladocera, such as *Daphnia*, the food in the intestine, within the peritrophic membrane, can be seen to be gradually moved back towards the rectum, and periodically portions of the faeces are expelled through the rectum. What is it that moves the food backwards? When there are suspended food particles, algae or detritus, in the water, *Daphnia* feeds continuously, and the swallowing of food by the gullet must result in pushing backwards food that is already in the intestine within the peritrophic membrane. But when there is no longer any food to be swallowed, water is still taken in rhythmically and continuously through the mouth, while food that is already in the intestine is gradually moved on and periodically defaecated. It looks, then, as if it is the water which is continuously pumped in by the gullet that pushes the food backwards in the intestine within the peritrophic membrane. At the same time the swallowed water must distend the intestinal wall. Defaecation would take place when the rising pressure in the intestine caused a reflex release of the contracted rectum, whereupon the contraction of the stretched intestinal muscles, and perhaps also the elasticity of the intestinal wall, would expel a portion of faeces.† Perhaps in other Crustacea, too, water swallowed through the mouth moves food backwards along the gut until rising pressure, due in part also to anal water intake, leads to defaecation.

MECHANISM OF ANAL AND ORAL WATER INTAKE

It is apparent that in order to contract rhythmically and effectively, the muscles of the intestine of Crustacea must be stretched by hydrostatic pressure. How is it, then, that the circular muscles of gullet and rectum, without being thus stretched, are able to pump water into the intestine, distending the muscles of the latter? The

* Miss Barbara M. Gilchrist has observed that in *Branchipus stagnalis* the contractions of the lobed anterior intestinal caeca are synchronized with another rhythmic movement: they contract each time that the gullet makes a peristaltic swallowing movement, thus driving the gulp of water or food along the intestine.

† In *Daphnia* defaecation is very rapid; when it occurs, intestinal antiperistalsis and rhythmic contractions of the caeca stop momentarily.

answer is that pharynx and rectum usually have radial dilator muscles (Weismann, 1874; Claus, 1876; Nowikoff, 1905; Humperdinck, 1924; Janisch, 1924; Ringel, 1924; Binder, 1932). As pointed out by Weismann (1874), the dilator muscles act as a suction pump, aspiring water to be forced into the intestine by the peristalsis or antiperistalsis respectively of the circular muscles of gullet or rectum. In aspiring the water, the dilators necessarily stretch the circular muscles and this enables them to pump the water inwards against pressure.

The action of the dilators of the gullet can easily be watched in living *Daphnia* and those of the rectum in Anostraca and in young *Triops*. In *Chirocephalus diaphanus*, although the gulps of water taken in by the rectum are small, rhythmic contractions of the dilator muscles are so strong that they move the body wall of the last abdominal segment. In *Daphnia* there are dilator muscles of the anus but not of the whole rectum (Binder, 1932). The action of these dilators in sucking water into the rectum may easily be watched under a low-powered microscope by putting *Daphnia* into a 5% solution of urethane, when the animals can be made to lie on their back. In *Daphnia* the rectal constrictor muscles are particularly well developed and are evidently strong enough to pump the aspired water into the intestine without themselves being stretched.

FATE OF THE WATER TAKEN INTO THE GUT

It is clear that most, or perhaps all, of the water taken in by prawns through the anus is expelled again through the same aperture at defaecation, or at pseudo-defaecation of water alone. In smaller Crustacea, with more frequent or even continuous rectal swallowing, water is certainly expelled again with the faeces, although we do not know what proportion this is of the water taken in. In some cases what appear to be astonishingly large quantities of water are pumped into the gut by the rectum when there are only infrequent defaecations that could get rid of the water. For instance, in a metanauplius of *Triops cancriformis* 324 rectal gulps of water were swallowed in $6\frac{3}{4}$ min. without a defaecation; in an individual of *Bythotrephes longimanus* uninterrupted rectal swallowing of water at the rate of 92 gulps per min. (and oral drinking at the rate of 22 per minute) was watched for 5 min. without there being a single defaecation; and in the small mysid *Sirella armata*, 8 mm. long, continuous rectal swallowing was watched for 15 min. with only one defaecation, the gulps being at the rate of 47 per minute.

The amount of water drunk through the mouth by Crustacea appears also to be large. What becomes of this water? In *Leptodora* there is sometimes *anti*peristalsis in the long midgut oesophagus and some of the water pumped into the stomach, from both mouth and anus, may then be vomited. But in *Daphnia*, *Diaphanosoma* and *Limnadia* I have never seen antiperistalsis in the gullet, and thus none of the water drunk continuously through the mouth is vomited. In most Crustacea other than Cladocera and *Limnadia* the gullet cannot be clearly observed. If the water taken in through the mouth is not usually vomited, is it got rid of through the anus? This cannot be its fate since the water coming in through the anus itself is, in many instances, apparently not all expelled by that path.

The water which we ourselves drink passes through the gut wall into the blood and is expelled from the body through the kidneys. In Crustacea the excretory organs, antennary or maxillary glands, must have a source of water too, and this water must enter the blood from either the gut or the gills. But is the very considerable quantity of water which is drunk by Crustacea through the mouth, with some at least of that swallowed by the rectum, largely passed through the gut wall and out of the body by the excretory organs? In favour of this is the following observation. If *Daphnia* is made to swim in a dilute solution of a dye, for instance bromo-thymol blue (Fox, 1948, p. 206) or nigrosin, after a few hours the dye can be seen to have accumulated in the liquid found in the anterior part of the intestine between peritrophic membrane and gut wall. The dye here is surprisingly concentrated and the process of accumulation is rapid. After *Daphnia magna* had been left overnight in a dilute solution of nigrosin, the concentration of dye in the lumen of the anterior part of the intestine was estimated. Animals were chosen which had no food in that part of the gut. The depth of colour of the dye in the gut of these animals, held lightly in a compressorium, was matched with that of the original solution in a flat-bottomed glass tube on the microscope stage (using a 2 in. objective), the height of solution in the tube being adjusted to give approximate equality of light absorption. The ratio of the height of solution in the tube to the internal diameter of the intestine showed that *Daphnia* had concentrated the dye about 250-fold. In nature the liquid between peritrophic membrane and gut wall of *Daphnia* is often bright green, the colour being due to a derivative of the chlorophyll of the algal food. The obvious explanation of this concentration of solutes in the gut lumen is that water is withdrawn through the gut wall from the solution. Perhaps the excretory organs of Crustacea require a particularly good flow of water and a further function of the more or less continuous oral drinking is to supply this water.

SUMMARY

In the majority of small and transparent Crustacea water can be seen to be pumped into the alimentary canal through the anus by rhythmic antiperistaltic swallowing movements of the rectum. This anal drinking is continuous in small species and in the young of larger species, but occurs in intermittent bursts in the adults of larger species.

In prawns the intermittent anal intake of water acts as an enema, for it occurs only at the time of defaecation, which is preceded by one or two dozen rapid rectal gulps of water. The continuous anal intake of water by smaller Crustacea acts likewise as an enema, being continuous because of the more frequent defaecations, due to the higher metabolism and therefore greater food requirements of small animals. The water acts as an enema as in man, stretching the gut-wall muscles until they contract.

In prawns the rectal swallowing of water initiates and maintains intestinal antiperistalsis, which moves the swallowed water forwards in the intestine towards the thorax. A further function of the anal intake of water is thus to stretch the gut-wall muscles until they contract antiperistaltically. This is comparable with the initiation and maintenance of the heart beat in molluscs by hydrostatic pressure.

In the past it has been thought that the rectal swallowing of water by Cladocera is respiratory. This opinion was apparently strengthened by experiments showing that a deficiency of dissolved oxygen increases the rate of rectal swallowing movements. These experiments have not been confirmed, and other reasons are given which make a respiratory function unlikely.

Two further possible functions of the intake of water through the anus, namely the collection of salts necessary for osmoregulation, and a hydrostatic maintenance of body turgor, are discussed, tested and rejected.

Water can be seen to be swallowed more or less continuously through the mouth, by rhythmic peristaltic movements of the gullet, in the majority of small and transparent Crustacea.

A function of this oral drinking appears to be the same as that of anal drinking, namely to stretch the muscles of the gut wall. In *Daphnia* the antiperistaltic contractions of the midgut wall, which mix food and digestive enzymes, seem to be maintained by the hydrostatic pressure of water pumped into the gut by both gullet and rectum, defaecation occurring when this pressure rises to a certain value.

A second function of the rhythmic oral drinking by *Daphnia* and perhaps other Crustacea is to force the food in the midgut back towards the rectum.

The gullet and rectum of Crustacea have dilator muscles inserted into the exoskeleton. These muscles suck in water through mouth and anus, and by stretching the circular muscles they enable the latter to pump the water into the gut.

Much more water is taken into the gut of Crustacea than makes its exit at defaecation. Evidence is given that this water passes through the gut wall into the blood and out of the body by way of the excretory organs.

This work was begun in June 1948 when it was observed while examining metanauplius larvae of Apus, recently rediscovered in England (Fox, 1949), that water is continuously taken into the gut through the anus. Much of the work has been done in the Zoology Department of Bedford College, London, with material collected locally or sent from Plymouth, and considerable parts of the study were made at the Istituto di Idrobiologia at Pallanza in 1948, 1950 and 1951, and at the Station Zoologique at Villefranche-sur-mer in 1949. My grateful thanks are due to the Directors of these two laboratories, the late Prof. Edgardo Baldi and Dr G. Trégouboff, for their hospitality and help, and to Prof. Vittorio Tonolli at Pallanza for much willing assistance. Dr Sandro Ruffo took great trouble in collecting *Palaemonetes* at Verona and sent the prawns alive to Pallanza, and the late Dr A. Pacaud kindly arranged for *Atyaephyra* to be collected for me from a river in the Loiret Department in France. *Penilia* and *Podon* were studied at the Stazione Zoologica at Naples, and phyllopods were hatched out in London from dried mud derived, with the help of friends, from various parts of the world.

REFERENCES

Bayliss, W. M. & Starling, E. H. (1899). The movements and innervation of the small intestine. *J. Physiol.* **24**, 99.

Biedermann, W. (1884). Beiträge zur allgemeinen Nerven- und Muskelphysiologie. XIV. *S.B. Akad. Wiss. Wien*, **89**, 69.

Binder, G. (1932). Das Muskelsystem von Daphnia. *Int. Rev. Hydrobiol.* **26**, 56.

Carlson, A. J. (1906). Comparative physiology of the invertebrate heart. V. *Amer. J. Physiol.* **16**, 47.

Claus, C. (1876). Zur Kenntniss der Organisation und des feineren Baues der Daphniden und verwandter Cladoceren. *Z. wiss. Zool.* **27**, 362.

Dubuisson, M. (1930). L'état actuel de nos connaissances sur le rôle de la distension du muscle cardiaque dans l'activité du cœur chez les Invértébrés. *Arch. int. Physiol.* **32**, 416.

Fox, H. Munro (1948). The haemoglobin of *Daphnia*. *Proc. Roy. Soc.* B, **135**, 195.

Fox, H. Munro (1949). On Apus: its rediscovery in Britain, nomenclature and habits. *Proc. Zool. Soc. Lond.* **119**, 693.

Fox, H. Munro (1952). Anal and oral water intake by Crustacea. *Nature*, **169**, 1051.

Fox, H. Munro & Johnson, M. L. (1934). The control of respiratory movements in Crustacea by oxygen and carbon dioxide. *J. Exp. Biol.* **11**, 1.

Fredericq, H. (1913). Recherches expérimentales sur la physiologie cardiaque d'Octopus vulgaris. *Arch. int. Physiol.* **14**, 126.

Fritsche, H. (1917–20). Schwankungen des osmotischen Druckes der Körperflüssigkeit bei Daphnia magna. *Int. Rev. Hydrobiol.* **8**, 22, 125.

Hotovy, R. (1938). Die Abhängigkeit des Sauerstoffverbrauches von der Temperatur und Körpergrösse bei Triops cancriformis B. *Zool. Anz.* **122**, 198.

Humperdinck, I. (1924). Ueber Muskulatur und Endoskelett von Polyphemus pediculus. *Z. wiss. Zool.* **120**, 621.

Jančařík, A. (1949). Contribution to the understanding of the intestine-breathing with the cladocere Moina rectirostris Leydig. *Věstn. čsl. zool. spol.* **13**, 185. (In Czech with English summary.)

Janisch, E. (1924). Der Bau des Enddarms von Astacus fluviatilis. *Z. wiss. Zool.* **120**, 1.

Johnson, M. L. (1936). The control of respiratory movements in Crustacea by oxygen and carbon dioxide. *J. Exp. Biol.* **13**, 467.

Koch, W. (1917). Der Herzschlag von Anodonta unter natürlichen und künstlichen Bedingungen. *Pflüg. Arch. ges. Physiol.* **166**, 281.

Lereboullet, A. (1850). Observations anatomiques et physiologiques. *Mém. Soc. Mus. Hist. nat. Strasbourg*, **4**, 211.

Nowikoff, M. (1905). Untersuchungen über den Bau der Limnadia lenticularis L. *Z. wiss. Zool.* **78**, 561.

Peters, F. (1938). Ueber die Regulation der Atembewegung des Flusskrebses Astacus fluviatilis Fabricius. *Z. vergl. Physiol.* **25**, 591.

Ranson, W. B. (1884). On the cardiac rhythm of Invertebrata. *J. Physiol.* **5**, 261.

Ringel, M. (1924). Zur Morphologie des Vorderdarmes (Schlund und Magen) von Astacus fluviatilis. *Z. wiss. Zool.* **123**, 499.

Siedentop, W. (1930). Ueber die Darmatmung von Leptodora Kindtii. *Arb. ung. biol. ForschInst.* **3**, 82.

Straub, W. (1901). Zur Physiologie des Aplysienherzens. *Pflüg. Arch. ges. Physiol.* **86**, 504.

Walshe-Maetz, B. M. (1952). Environment and respiratory control in certain Crustacea. *Nature, Lond.*, **169**, 750.

Weismann, A. (1874). Ueber Bau und Lebenserscheinungen von Leptodora hyalina. *Z. wiss. Zool.* **24**, 349.

Weymouth, F. W., Crismon, J. M., Hall, V. E., Balding, H. S. & Field, J. (1944). Total and tissue respiration in relation to body weight. A comparison of the kelp crab with other crustaceans and with mammals. *Physiol. Zoöl.* **17**, 50.

Zeuthen, E. (1947). Body size and metabolic rate in the animal kingdom. *C.R. Lab. Carlsberg*, **26**, 20.

DIAPAUSE IN *LUCILIA SERICATA* (MG.) DIPTERA

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(With One Text-figure)

INTRODUCTION

It is now well established that unfavourable environmental conditions such as cold or excessive moisture acting on larvae of the blowfly *Lucilia sericata* may produce diapause. Further, Cousin (1932) showed that adults of this species subjected to abnormal conditions produced eggs which, if not sterile, had a high mortality and the ensuing larval stage was longer than that of normal larvae.

In the course of several years' work on *L. sericata* one of the present writers has often observed that many larvae collected in the field towards the end of the blowfly season enter diapause in spite of completing their larval feeding period under laboratory conditions favouring pupation. Larvae on small carcasses might experience adverse environmental conditions prior to collection, but this is not necessarily true for larvae taken off sheep suffering from myiasis. In the latter case, even in autumn, the larval environment is suitable for normal development. It would seem, therefore, that the diapause which occurred might be of maternal origin. This view was given further support by some preliminary experiments carried out in 1949 by one of the writers (J.B.C.) in collaboration with Miss B. A. Thurston. It was found that egg batches from wild flies caught in late summer, even when reared under laboratory conditions favouring normal development, gave a high proportion of diapausing larvae. The hypothesis was therefore carefully tested in the blowfly seasons of 1950–51 by comparing the diapausing tendency of field-caught *L. sericata* with that of laboratory controls ovipositing at the same time.

MATERIAL AND METHODS

Female *L. sericata* were captured under field conditions, either in traps baited with the standard hydrogen sulphide-mercaptan attractant (Cragg & Thurston, 1950) or by being attracted to sheep (Cragg, 1950). The majority of the flies attracted to sheep were caught and taken to the laboratory, each in a separate $7{\cdot}5 \times 2{\cdot}5$ cm. tube containing a small lock of moist sheep wool. Some flies oviposited on this wool but most oviposited on meat in the laboratory. Occasionally flies were induced to lay on sheep, the egg batches being transferred to the laboratory. Control flies taken to the field station in tubes and then brought back to the laboratory gave larvae which behaved in the same way as those produced by the normal laboratory culture; thus the circumstances of collection did not cause diapause.

Both control and field-caught flies were kept individually in lamp glasses 16 cm.

high and 12 cm. diameter, the tops being covered with muslin and the bases placed in trays containing damp sawdust. Each lamp glass contained a water vessel, sugar and meat. These lamp-glass cages were kept in a constant temperature room at 26° C. and 60–70 % R.H., and were illuminated for approximately 8 hr. per day by fluorescent lighting.

Egg batches were hatched on small pieces of rearing medium at 27° C. and 100 % R.H. After hatching, approximately 150 1st-instar larvae were transferred to 500 ml. conical flasks containing 55 g. of medium. Twenty-four hours later the medium was covered with a thick layer of slightly damp sawdust to absorb excess moisture. Rearing was carried out at 26° C. When larvae began to leave the medium (5–6 days after egg-laying) they were hand-sorted into honey-jars 10 cm. deep and 7 cm. diameter containing damp sawdust, to complete the larval and pupal stages at 22° C.

The rearing method and the breeding medium were modified from Lennox (1939) and Hill, Bell & Chadwick (1947). The medium contained: 100 ml. fresh slaughter-house blood, 6·7 g. Brewer's yeast, 0·3 g. potassium (monobasic) phosphate and 0·5 g. agar-agar. It was autoclaved for 15 min. at 15 lb. pressure.

Controls were taken from a laboratory culture started in late July 1950 from larvae collected off sheep. As far as possible, equal numbers of field-caught and control flies were used for each set of observations. Only field-caught flies ovipositing within 24 hr. of capture were used; in most cases eggs were laid within 2 hr.

Batches of *L. sericata* larvae, even from well-established laboratory cultures, usually give a small percentage of diapausing larvae (2–10 %). In the present investigation, therefore, the following criterion was used to measure diapause. A batch was considered normal if it showed 50 % pupation within 14 days of completing feeding. Those which failed to reach this level were regarded as diapausing batches. Of 147 normal batches 135 showed 80 % pupation within 10 days, whereas so-called diapausing batches never reached this level in less than 30 days and often required more than 100 days.

RESULTS

(i) 1950. Table 1 and Fig. 1 show that the incidence of diapause in larvae of field-caught flies increased as the blowfly season approached its end. Some diapause occurred among control batches and the difference between control and field groups was only significant at the 0·01 level in the last sample. Because of the small number of flies in both control and field-caught groups the Fisher-Yates test of significance described by Finney (1948) was used.

(ii) 1951. As is clear from the table the trend shown in the 1950 results was established with a high degree of significance in 1951.

(iii) Simmonds (1948) has shown that in *Spalangia drosophilae* and *Cryptus inornatus* the age of the mother can influence the amount of diapause. Since the *Lucilia* population at the end of the season may contain a high proportion of old flies, the increase in diapause might therefore be related to age. This possibility was tested by comparing larvae from 2–6-week-old control flies; ten batches were

Table 1

Periods when egg batches were laid	Control flies		Wild flies		χ^2	P
	No. giving normal batches	No. giving diapause batches	No. giving normal batches	No. giving diapause batches		
A. 1950 results						
26 July–9 Aug.	5	1	9	1	0·15	0·7*
10–20 Aug.	None	None	10	3	—	—
21 Aug.–4 Sept.	2	0	7	4	1·05	0·3*
5–19 Sept.	6	1	5	6	2·91	0·1*
20 Sept.–2 Oct.	8	2	1	3	3·76	0·05*
2–17 Oct.	8	0	4	6	7·20	0·01*
B. 1951 results						
20 July–3 Aug.	8	0	7	1	1·07	0·3*
4–17 Aug.	28	0	20	4	5·06	>0·02
18–31 Aug.	35	0	17	7	11·5	<0·001
1–14 Sept.	21	0	19	5	4·92	<0·05
15–28 Sept.	23	0	9	11	16·9	<0·001
29 Sept.–12 Oct.	20	0	11	12	14·5	<0·001
13–19 Oct.	12	0	3	8	13·4	<0·001*

Normal batches of larvae. Those which achieved 50% pupation within 14 days of finishing feeding.
Diapausing batches of larvae. Those taking more than 14 days to reach 50% pupation.
* Significance calculated according to Finney (1948).

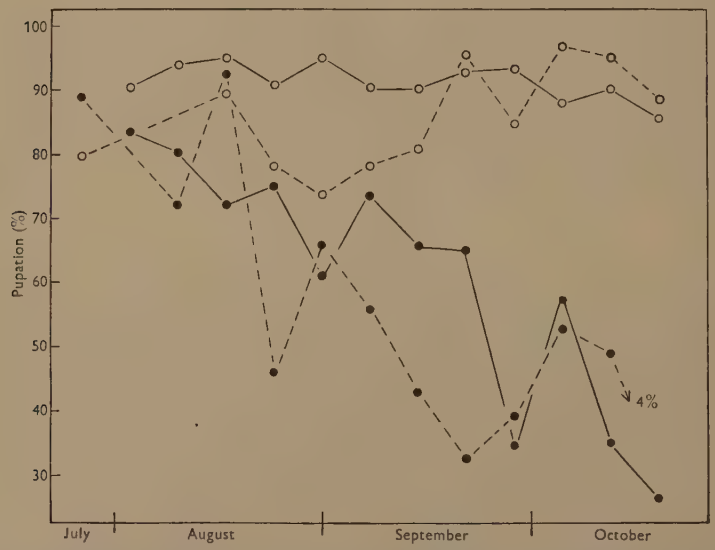

Fig. 1. Open circles show average percentage pupation of control batches and closed circles the pupation in batches from field-caught flies. 1950 results are shown by broken lines, 1951 results by solid lines.

compared, no diapause being recorded in either group. Thus it is unlikely that age has a significant effect on the induction of diapause.

(iv) In 1951, of twenty-one field-caught flies whose first egg batches gave diapausing larvae, sixteen gave normal egg batches after 10–16 days in the laboratory. Of fourteen whose first egg batch was normal, two gave diapausing larvae in subsequent batches.

(v) There was no obvious correlation between the fluctuations shown in the figure and any one weather measurement.

DISCUSSION

Cousin (1932) and Mellanby (1938) have shown that unfavourable conditions acting directly on *L. sericata* larvae can induce diapause. The present investigation makes it clear that diapause in this species may also be of maternal origin. The physiological conditions inducing this type of diapause ceased to operate in most flies kept under laboratory conditions for 10–16 days. However, out of fourteen flies whose first egg batches were normal, two subsequently gave diapausing batches. This delayed occurrence of diapause suggests that the factors leading to its maternal induction take some time to affect developing oocytes.

Cousin claims to have reared *L. sericata* through eighty generations without the occurrence of diapause and she states that: 'Tous les individus des pontes successives ont été suivis d'un bout à l'autre de leur developpement.' Such a complete absence of diapause has not been realized in the course of several year's work in Great Britain. Here, even under optimal conditions, a small percentage of larvae may enter diapause. This might indicate that some larvae occur for which normal rearing conditions are not favourable. Another possibility is that in certain larvae the physiological state necessary for pupation is only slowly acquired.

Given suitable rearing conditions for both adults and larvae, diapause in *L. sericata* never shows a cyclical pattern such as Theodor (1934) described for *Phlebotomus papatasii*. It is, instead, clearly related to some seasonal change, and in view of the importance of temperature and photoperiod in other organisms (see reviews by Lees, 1950, and Andrewartha, 1952) their effect on *Lucilia sericata* adults should be studied. Dickson (1949) has shown that the photoperiod does not influence the amount of diapause in *L. sericata* larvae, but considering the normal habitat of blowfly larvae this result is not unexpected.

Diapause is often a method of surviving adverse conditions, particularly winter cold. The occurrence of maternal-induced diapause in *L. sericata* and perhaps in other blowflies over-wintering in the so-called prepupal state, ensures that the majority of larvae produced at the end of the blowfly season enter diapause in spite of the possible chance occurrence of ground conditions favouring pupation.

SUMMARY

Data collected over a period of 2 years have shown that diapause in 3rd-instar larvae of *Lucilia sericata* (Mg.) may be of maternal origin and that its incidence gradually increased in the latter part of the blowfly season. The age of female flies

has no significant effect on the amount of diapause. The capacity to give diapausing batches was lost by the majority of flies kept under standard laboratory conditions for 10–16 days.

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REFERENCES

Andrewartha, H. G. (1952). Diapause in relation to the ecology of insects. *Biol. Rev.* **27**, 50–107.
Cousin, G. (1932). Étude expérimentale de la diapause des insects. *Bull. biol.* Suppl. 15, 1–341.
Cragg, J. B. (1950). The reactions of *Lucilia sericata* (Mg.) to various substances placed on sheep. *Parasitology*, **40**, 179–87.
Cragg, J. B. & Thurston, B. A. (1950). The reactions of blowflies to organic sulphur compounds and other materials used in traps. *Parasitology*, **40**, 187–94.
Dickson, R. C. (1949). Factors governing the induction of diapause in the oriental fruit moth. *Ann. Ent. Soc. Amer.* **42**, 511–37.
Finney, D. J. (1948). The Fisher-Yates test of significance in 2×2 contingency tables. *Biometrika*, **35**, 145–56.
Hill, D. L., Bell, V. A. & Chadwick, L. E. (1947). Rearing of the blowfly, *Phormia regina* Mg., on a sterile synthetic diet. *Ann. Ent. Soc. Amer.* **40**, 213–16.
Lees, A. D. (1950). The physiology of diapause. *Sci. Progr. Twent. Cent.* **38**, 735–42.
Lennox, F. G. (1939). The development of a synthetic medium for aseptic cultivation of larvae of *Lucilia cuprina*. *Pamphl. Coun. Sci. Industr. Res. Aust.* **90**, 1–24.
Mellanby, K. (1938). Diapause and metamorphosis of the blowfly *Lucilia sericata* Mg. *Parasitology*, **30**, 392–402.
Simmonds, F. J. (1948). The influence of maternal physiology on the incidence of diapause. *Philos. Trans.* B, **233**, 385–414.
Theodor, O. (1934). Observations on the hibernation of *Phlebotomus papatasii* (Dipt.). *Bull. Ent. Res.* **25**, 459–72.

VARIOUS TYPES OF GHOSTS DERIVED FROM HUMAN RED CELLS: HEAT FRAGMENTATION AND PHASE OPTICS STUDIES

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(With Two Text-figures)

This investigation started with the incidental observation that a slight modification in the method used for preparing ghosts from human red cells results in a remarkable difference in one of the properties of the ghost; more specifically, when ghosts are prepared by the addition of large volumes of water, they are not fragmented by heat unless a certain concentration of salt is present (Ponder, 1951). Extension of these observations, made possible by phase-contrast optics, has led to the idea, already suggested by electron microscope studies, that a large number of kinds of ghost, each with its own characteristic properties, can be prepared from a single kind of red cell. This is a very different idea from the usual one that the ghost is a 'residue' which, apart from small differences in chemical composition, is essentially the same whether it is obtained from one kind of red cell or from another.

While the purpose of this paper is to illustrate, rather than to describe exhaustively, the multiplicity of the kinds of ghosts which can be obtained (*a*) by haemolysis in hypotonic media, (*b*) by haemolysis in hypotonic media followed by 'reversal of haemolysis', (*c*) by haemolysis by freezing and thawing, and (*d*) by haemolysis with various lysins, it is convenient to extend the illustrations to include descriptions of the methods by means of which the various kinds of ghost can be obtained reproducibly.

All the observations to be described were made with a phase-optics system which employs an oil immersion (97 ×) objective, a 5 × eyepiece, and a green filter. Direct observation of individual fragmenting cells can also be made with phase optics and the heating chamber already described (Ponder, 1950*a*). A chess-board micrometer mounted in the eyepiece, the squares corresponding to about 4 μ, enables estimates of the sizes of red cells and of ghosts to be made.

The technique for producing and studying fragmentation has already been described (Ponder, 1950*a*, *b*).

I. GHOSTS PRODUCED BY HAEMOLYSIS WITH WATER

These ghosts are prepared by adding washed and incubated* human red cells to large volumes of water, allowing the haemolysate to stand at 4° C., and separating the ghosts by centrifuging (Ponder, 1951). The shape of the ghosts prepared by

* The incubation of the washed red cells at 37° C. produces an effect which is quite obscure. The opacity of the haemolysate made from incubated cells increases with the length of the incubation, and the yield of ghosts is increased correspondingly.

this method depends on how long they have been allowed to stand, either in contact with the haemolysate at 4° C., or separated from it. If less than 24 hr. have elapsed since the commencement of the preparation, the ghosts, which can be seen very clearly with the phase optics, are biconcave or cup-shaped disks. If they have stood for longer times, they tend to be spherical, and may even show spontaneous fragmentation.

(1) *Concentration of* NaCl *needed for heat fragmentation.* The ghosts do not fragment in the medium in which they are suspended, even if they are heated for several minutes to 80° C. If NaCl is added, heating to 52° C. for 3 min. breaks them into many fragments of varying sizes (cf. Ponder, 1950*a*). By varying the concentration of NaCl, it can be shown that a concentration of between 0·05 g./100 ml. and 0·1 g./100 ml. (0·0085–0·017 M) is critical; at concentrations above this, fragmentation occurs almost independently of the concentration, while at lower concentrations there is virtually no fragmentation at all. The salt must be present during the period of heating; adding it, washing, and then heating does not result in fragmentation, and heating the ghost suspension and then adding salt is equally ineffective.

(2) *Effect of other salts, etc.* The effect of KCl, LiCl, $CaCl_2$, Na_2SO_4, NaF, NaBr and NaI, all in 0·017M concentration, is indistinguishable from that of 0·017M-NaCl. Fragmentation also occurs in systems containing 0·034M glycine or sucrose, although its extent is usually less than in systems containing the same concentration of NaCl, KCl, LiCl, etc. Changes in pH, brought about by phosphate buffers, have a slight effect on the fragmentation observed when the ghosts are heated to 52° C. in 0·017M-NaCl, the fragmentation being somewhat greater at pH 8·6 than at pH 5·6.

(3) *Effect of saponin.* Ghosts prepared in a large volume of water and exposed to high concentrations of saponin dissolved in 0·1 % NaCl are not fragmented when heated for 3 min. to 55° C. If the concentration of saponin is less than about 200 μg./ml., on the other hand, fragmentation occurs on heating, just as it does in 0·1 % NaCl.

(4) *Effect of serum and of serum albumin.* Ghosts prepared in large volumes of water do not undergo a disk-sphere transformation between slide and cover-glass (Ponder, 1942), and the addition of serum or of 1 % serum albumin produces little immediate change of shape. The ghosts in the presence of either serum or serum albumin, however, are less smooth than in water alone; this may be the result of an osmotically produced volume decrease, or may be related to the spontaneous fragmentation which is seen in the preparations after the lapse of some hours.

When the ghosts suspension containing equal volumes of serum is heated, there is extensive fragmentation with the production of long myelin forms. The extent of the fragmentation depends on the temperature and duration of the heating, and is the same even if 1 % NaCl is added to the preparation in addition to the serum, which itself supplies the electrolyte necessary for fragmentation. When 1 % salt-free serum albumin is added to the ghosts suspension, either with or without the addition of 1 % NaCl, the fragmentation which occurs during heating in the presence

of salt is suppressed almost entirely; the ghosts of the heated suspension are shrunken, distorted and agglutinated, but they are not fragmented as they are in systems containing more than 0·1 % NaCl but no albumin. Myelin-form formation, however, is much more conspicuous than in the latter systems, although it differs from the myelin-form formation in systems of ghosts containing serum, the forms being short and shaggy instead of long and thin.

These effects may be summarized by saying that the addition of serum to suspensions of ghosts prepared in large volumes of water results in their being fragmented by heat and in the production of one type of myelin form, whereas the addition of serum albumin protects the ghosts from heat fragmentation and leads to the production of a morphologically different type of myelin form.

II. GHOSTS RESULTING FROM 'REVERSAL OF HAEMOLYSIS'

Ghosts can be prepared by haemolysing human red cells in volumes of water which vary from about 5 to over 100 vol., the watery haemolysate being brought, after various lengths of time at various temperatures, to isotonicity by the addition of salts ('reversal of haemolysis'). By varying the kind of salt added, the reversal can be brought about at various values of pH, etc. The ghosts in such systems can be centrifuged down, examined, heated, frozen, and so on, and the properties of the ghost vary with the method of preparation.

(*a*) *Phosphate systems*

A series of solutions which, when diluted to a concentration of 0·1 M, give pH values from 5·5 to 7·3, can be prepared from mixtures of 1·5 M-NaH_2PO_4 and 1·5 M-Na_2HPO_4. Suspensions of red cells haemolysed in 15 (or more) vol. of water can be brought to isotonicity at any pH within the range by adding 1 (or more) vol. of these solutions; if the cells are haemolysed with less than 15 vol. of water, the phosphate mixtures are diluted proportionally before being added to the haemolysate. The pH of the systems is limited by the low solubility of Na_2HPO_4, but one can reach pH 8·0 by using 0·6 M-Na_2HPO_4 and adding proportionately larger volumes of the buffer mixtures to bring about reversal of haemolysis.

(1) *Freshly prepared ghosts.* The red cells of heparinized human blood can be quickly washed three times with saline to make a suspension of the same volume concentration as that of blood, and then haemolysed in 15 vol. of water at 25° C. If the haemolysate is allowed to stand for 15–20 min., 1 vol. of 1·5 M phosphate buffer being added at the end of the period of standing, ghosts can be thrown down (2×10^3 g. for 15 min.). These ghosts are flat, coarsely crenated disks, which do not show spontaneous fragmentation in times up to 2 hr. If the pH of the added buffer is 5·5, heating to 53–55° C. converts the cells into spheres but does not fragment them. If the pH is higher than about 6·5, the cells become spherical and some of them break into two or three fragments; there is, however, no extensive fragmentation and very little appearance of myelin forms.

(2) *Ghosts prepared from cells after standing.* If the same suspension of human

red cells is kept at 4° C. for 12 hr. or more, repetition of the same process as that described above results, at all values of pH, in a yield of thin, coarsely and finely crenated ghosts. The fine crenations are most conspicuous at the edges. These ghosts have no marked tendency to spontaneous fragmentation, although some of the small crenations may break off to form tiny beads at, or separated from, the cell edges. If the pH of the added buffer is 5·5, heating to 53–55° C. results in spherical forms which do not fragment, but if the pH is 6·2 or more, heating results in the ghosts breaking into innumerable tiny spherical fragments accompanied by many long myelin forms. Comparison of this result with that obtained with freshly prepared ghosts shows that heat fragmentation is favoured by an increase in pH and also by the time during which the preparation stands, i.e. by the 'age' of the preparation.

(*b*) *Veronal systems*

The buffer system described by Michaelis (1931) has the advantage that it gives pH's from 2·6 to 9·6, the concentration of the salts (sodium acetate, sodium veronal, NaCl and HCl) being such as to have a constant ionic strength at all values of pH, and to be isotonic with plasma. The buffer system is somewhat limited by its low solubility at low pH. Mixtures above pH 8 can be prepared with a tonicity of 5 times that of 1 % NaCl; mixtures at pH less than 8 are prepared with a tonicity of twice that of 1 % NaCl. Reversal of haemolysis is brought about by adding 1 vol. (above pH 8), or 4 vol. (below pH 8) of the buffers to freshly prepared washed red cell suspensions (volume concentration 0·4) haemolysed with 4 vol. of water.

Between pH 5 and 8, the ghosts produced by the reversal are disks with a moderate amount of coarse crenation. At pH 4 they tend to be smooth and cup-shaped, with an accentuated rim; at pH 9 they tend to be crenated spheres. The effect of heating to 53° C. for 3 min. depends on the pH of the added buffer. At pH 4, there is no fragmentation, shrunken irregularly-shaped ghosts of from 4 to 6 μ in diameter being aggregated into masses. At pH 5, the ghosts are dull objects with almost no surface reflectivity; fragmentation apparently takes place, but the fragments either do not separate completely or stick to each other after separation in small irregular clusters about the size of a red cell. At pH 6, there is fragmentation into spheres which have a low surface reflectivity; many minute myelin forms can be seen at their edges, and their dull appearance may be due to the surface being covered with small myelin forms. As the pH is increased, the fragmentation and the development of myelin forms increases, so that many of the fragments are flat dull objects to which extruded myelin forms are attached and which break down, as time goes on, into networks of myelin forms of many shapes and sizes.

As compared with the results obtained with freshly prepared ghosts after reversal of haemolysis with phosphate, the formation of myelin forms in veronal systems is quite remarkable; it resembles the myelin-form formation which is seen in phosphate systems only if the cells have stood at low temperatures for 24–48 hr. The appearance of the non-reflective masses of fragments after heating at pH 5, and the extensive fragmentation and myelin network formation after heating at pH 8 and 9 are peculiar to the veronal systems.

(*c*) CO_2-*saturated water systems*

Ghosts made by Parpart's (1942) method, which consists in haemolysing washed red cells with water, adding them to a large volume of cold CO_2-saturated water, and washing repeatedly with this same medium, are almost Hb-free and tend to agglutinate into masses. Seen singly with phase optics, they are very thin and tenuous bodies, roughly circular, and flat or cup-shaped. They do not have the distinct rim which ghosts prepared in large volumes of water have, nor do they have as great a volume.

On heating them to between 52 and 56° C., either in the medium in which they are suspended or with added 1 % NaCl, no fragmentation is observed. There is a great deal of agglutination into masses of distorted objects, which, since they have a diameter of about 5 μ, are probably roughly spherical. The surfaces of these ghosts are covered with shaggy irregularities which are probably myelin forms and which no doubt contribute to the great adhesiveness between the ghosts. When the preparation is pressed on, many of the myelin forms become dislodged, and float in the medium as tiny droplets or fine threads. Except for the appearance of the myelin forms, the ghosts formed in CO_2-saturated water resemble those found after phosphate reversal of haemolysis by phosphate at pH 5·5.

There is independent evidence of a difference between the ghost formed by haemolysis in large volumes of water and those which are subsequently flocculated out with CO_2. The electrophoretic mobility of the latter is only about 80 % of that of the former (Furchgott & Ponder, 1941), and the latter do not disintegrate into myelin forms when lyotropic agents are added (Furchgott, 1940).

III. EFFECTS OF FREEZING AND THAWING

While red cells which are frozen to −20° C. for 12 hr. and then thawed undergo haemolysis, they do not fragment. The form of the ghost is that of a sphere. Heating the ghosts to between 50 and 60° C. does not fragment them.

Ghosts prepared in large volumes of water are not fragmented by freezing and thawing, provided that they are relatively fresh, i.e. that not more than 72 hr. has elapsed since the beginning of the process by which they are prepared. This is true whether the form of the ghost is that of a disk or that of a sphere. After freezing and thawing, however, many of the watery ghosts show a vacuolation phenomenon which looks very similar to that described by Dervichian & Magnant (1947) in haemoglobin-nucleinate-myristylcholine coacervates. Sometimes one or two, but more often several round or oval vacuoles appear in the substance, or in the interior, of the ghost, which itself is often so large (12 μ in diameter and apparently spherical because it is never seen on edge) as to suggest that either imbibition or coalescence phenomena are involved. Heating the ghosts of these frozen and thawed preparations does not produce fragmentation, either in the absence or in the presence of salt.

If the ghosts are not relatively fresh, i.e. if they have been kept for more than 96 hr. either in the large volume of water in which they are prepared or even

separated from it, the discoidal form tends to be replaced by the spherical form, and both tend to undergo spontaneous fragmentation. Freezing and thawing of such ghosts increases the amount of fragmentation considerably. Heating the ghosts, either with or without salt, however, does not increase the fragmentation appreciably. The conclusion is that freezing and thawing, whether of red cells or of watery ghosts, produces a ghost which is not fragmented by heat. The properties of the watery ghost must therefore be changed, by freezing and thawing, into those of the ghost produced from the red cell by freezing and thawing.

IV. GHOSTS PRODUCED BY THE ACTION OF HAEMOLYSINS

The ghosts resulting from the action of different haemolysins on human red cells are different in appearance and in their ability to be fragmented by heat. Their appearance and behaviour are probably dependent on the concentration of lysin used, but the following descriptions will illustrate the principal differences observed. In each case the haemolytic system was composed of 1 ml. of washed red cells of fresh human blood, suspended in 1 % NaCl in a volume concentration of 0·4, with the addition of 4 ml. of the lysin dissolved in saline in the concentration given. The concentrations were such as would produce complete lysis within 5 min. at 25° C.; the results are accordingly those for systems containing the lysins in concentrations which are neither very great nor very small. The ghosts are separated by centrifuging, a little saline being used to suspend them. The descriptions of the effects of heating refer to a 3 min. heating to 53° C.

(1) *Saponin* (1 mg./ml.). The ghosts are pale, round or spheroidal bodies measuring 5–6 μ in diameter. Their edges are sharp; there is no substantial 'rim' which would suggest that the surface of the cell encloses a considerable volume. No spontaneous fragmentation or development of myelin forms occurs. After heating, there is no noticeable change in shape, and there is no fragmentation. If the concentration of saponin in the system is reduced to about 200 μg./ml., however, fragmentation takes place on heating (see above).

(2) *Digitonin* (0·1 mg./ml.). The ghosts are discoidal, cup-shaped, and sometimes biconcave bodies, very thin, but with a clearly observable 'rim'. The ghosts tend to stick together in masses. Heating results in extensive fragmentation into discrete, round fragments of all sizes, down to those of a 'dust'. No long myelin forms, however, are to be seen. When the concentration of digitonin in the system is some 5 times greater, however, heating does not produce fragmentation. As in the case of saponin, the occurrence or non-occurrence of heat fragmentation depends on the concentration of the lysin.

(3) *Sodium dodecyl sulphate* (1 mg./ml.). The ghosts are disks, usually cup-shaped, but occasionally biconcave, not so thin as the ghosts in digitonin systems, and with a clearly visible 'rim'. They measure about 8 μ in diameter, and from 0·5 to 1·5 μ in thickness. After heating, the disks are replaced by spheres of various sizes (2–4 μ in diameter). These do not appear to be the result of a fragmentation process, but rather of the disks of varying volumes having assumed the spherical form. It is tempting to think of these spheres as being composed of the 'fixed

framework' of the ghost in the form of a solid mass; since the diameters of the spheres vary from 2 to 4 μ, their volumes vary from about 4 μ^3 to about 30 μ^3, and something between these two values would be a likely enough figure for the volume of the 'fixed framework' of the ghost. Many of the small spheres have small, shaggy myelin forms attached to their surfaces.

(4) *Sodium oleate* (1 mg./ml.). The ghosts are small spheres with a diameter of between 2 and 4 μ. After heating they are replaced by many tiny spheres with diameters between 1 and 2 μ. The change in size probably involves a fragmentation or a disintegration phenomenon, and the surfaces of many of the little spheres is irregular and covered with minute myelin forms.

As an extension of these observations, suspensions of ghosts were prepared by the method described in § I, and lysins in various concentrations were added. After observing the changes produced by the lysin, each system was heated for 3 min. to 53° C. and re-examined. The phenomena observed were much the same as those observed in systems containing red cells and lysins. Ghosts prepared in large volumes of water, although discoidal and readily fragmented by heat when salt is present, are not fragmented by heat when treated with saponin in saline in concentrations greater than about 200 μg./ml. Fragmentation is also prevented by treating the watery ghosts with concentrated solutions of digitonin, although heat fragmentation and myelin-form formation occurs if the concentration of digitonin is less than about 50 μg./ml. Treatment with sodium dodecyl sulphate converts the discoidal watery ghosts to small spheres which are exceedingly difficult to see even with phase optics; heating of these results in still smaller spheres, probably as a result of shrinkage or the loss of myelin material into the surrounding fluid. Treatment of watery ghosts with oleate results in the formation of spheres, some with myelin forms attached; heating of these result in still more myelin-form formation, but not in any real fragmentation.

The generalization which seems to hold is that the ghosts produced by the action of these haemolysins, or by the action of these haemolysins on the ghosts produced by water, may be either discoidal or spherical, but that they are not fragmented by heat if the concentration of the lysin is greater than a certain critical concentration. In all these systems, spontaneous fragmentation and myelin-form formation are not at all conspicuous, at least when the cells and ghosts are derived from freshly drawn heparinized blood.

V. RÉSUMÉ AND DISCUSSION

It will be helpful to condense the foregoing detailed descriptions into a table (Table 1) and a list of conclusions. The table will show how the shape of the ghost and the effect of heat in producing fragmentation, myelin forms, and shape changes depends on the method of preparation; the list which follows will summarize the principal generalizations which can be arrived at from the observations:

(1) The ghost which results from haemolysis in large volumes of water does not fragment when heated unless one of the number of substances, which includes NaCl, glycine and sucrose, is added in sufficient concentration.

(2) This fragmentation is prevented by serum albumin.

(3) Fragmentation of ghosts by heat is favoured by a high pH and by increasing 'age' of the preparation; this seems to be true of ghosts however formed, provided that they fragment at all.

Table 1

		Fragmentation	Myelin forms	Shape change with heating
I. Water (disks and cups)	Salt-free	No	No	—
	+salt	Yes	Yes	—
	+saponin	No	No	—
	+serum	Yes	Yes (long)	—
	+serum albumin	No	Yes (short)	—
	Freezing and thawing	No	No	—
II. Reversal of haemolysis (disks and cups)	Phosphate: Fresh pH 6	No	No	Spheres
	pH 6	Slight	No	Spheres
	Old pH 6	No	No	Spheres
	pH 6	Yes	Yes	—
	Veronal pH 4	No	No	Shrinking
	pH 6	Yes	Yes (short)	—
	CO_2	No	Yes (short)	Irreg. with shrinking
III. Freezing and thawing	(Spheres)	No	No	Vacuolation phenomena
IV. Lysins*	Saponin (disks, cups)	No	No	—
	Digitonin (disks, cups)	No	No	—
	C-12 (disks, cups)	No	Yes (small)	Spheres
	Sodium oleate (spheres)	?	Yes (minute)	Spheres, very small

* These effects are functions of lysin concentration.

(4) Different methods of preparation result in the formation of ghosts with different properties, e.g. the ghosts formed in CO_2-saturated water have a low electrophoretic velocity, and do not fragment when heated; the ghosts formed in veronal systems of pH greater than 6 develop extensive myelin forms when heated.

(5) Ghosts prepared by freezing and thawing do not fragment when heated, and freezing and thawing of ghosts produced by haemolysis in water renders them non-fragmentable by heat even in the presence of salt.

(6) When ghosts are formed by the action of chemical lysins such as saponin and the soaps, their shape may be either discoidal or spherical, but, if the concentration of lysin is great enough, they do not fragment when heated.

To begin with the first observation on the list, an obvious possibility is that the fragmentation or non-fragmentation of ghosts, on being heated in the systems described, is determined by their shape, discoidal ghosts, like discoidal red cells, being easily fragmented, whereas spherical ghosts, like spherical red cells, are fragmented by heat with greater difficulty or not at all (Ponder, 1950*a*). This simple idea is untenable in view of the fact that ghosts prepared in large volumes of water

are discoidal if less than about 24 hr. elapse between the beginning of the process of preparation and the separation of the ghosts from the water, and that these ghosts do not fragment on heating to 53° C. unless salt is added. If prepared by reversal of haemolysis by phosphate, indeed, discoidal ghosts do not fragment even if salt is present provided that they are freshly prepared from fresh red cells.

Another simple idea is that fragmentation occurs only when the ghost contains a considerable concentration of surplus Hb, i.e. that the ghost prepared in a large volume of water does not fragment when heated because the concentration of Hb, although considerably in excess of that in the surrounding medium, is not great enough; a still greater concentration of surplus Hb would be, on this hypothesis, the necessary condition for fragmentation, and would be produced only when the volume of the ghost is reduced by the addition of NaCl or other osmotically active substances. It is certainly true that the processes which result in ghosts which are not fragmentable by heat (e.g. lysis by saponin or digitonin, or lysis by freezing and thawing) are processes which leave very little surplus Hb.* If the concentration of surplus Hb is a factor which enters into the situation, the way in which it does so is obscure. On the view that fragmentation by heat occurs at an unstable stage in the transition from the initial state of the ghost ultrastructure to its final state of a viscous fluid (Ponder, 1950*a*), the surplus Hb, which is now regarded as a component of a lipoprotein-Hb complex (Ponder, 1951), could possibly supply the viscous element, but the idea that the concentration of surplus Hb determines the ease and extent of fragmentation is not entirely adequate as a simplifying hypothesis even when it is combined with the idea of the discoidal shape as a determining factor. As has already been remarked, ghosts prepared from fresh red cell suspensions by reversal of haemolysis, do not fragment when heated even though they are disks and contain relatively large amounts of surplus Hb. Again, ghosts prepared by reversal of haemolysis by the addition of phosphate or veronal buffers do not fragment when heated if the pH of the system is sufficiently low, although they are discoidal and although they contain various and considerable amounts of surplus Hb. Finally, the hypothesis does not account for the action of serum albumin in preventing heat fragmentation which would occur in its absence,† nor can it be extended to cover subsidiary occurrences such as the appearance or non-appearance of myelin forms and their variety, or the effect of 'ageing'. Factors in addition to those of shape and surplus Hb concentration seem to be involved, and the situation is apparently too complicated to be explained in a simple way.

Although not entirely satisfactory as an explanation, the idea that fragmentation of ghosts depends largely on the concentration of surplus Hb is interesting because it leads to a new set of relations. The hypothesis that fragmentation occurs at a stage

* The higher concentrations of saponin liberate the surplus Hb from the ghost, with the result that the system becomes translucent. A similar translucence is observed in a heated suspension of ghosts prepared by haemolysis in a large volume of water and in which there is no fragmentation; this can be contrasted with the opaque appearance of the heated suspension of ghosts in 0·1 % NaCl, in which there is extensive fragmentation. The translucence is also seen in suspensions of ghosts prepared by freezing and thawing.

† Serum and serum albumin also affect the heat fragility of red cells in a complex way (Ponder, 1950*a*).

in a transition towards a viscous fluid can be restated by saying that fragmentation depends on the plasticity or cohesiveness of the cell as a whole. Now plasticity may be expected to have a special value corresponding to some special value for the cell volume, for if the cell volume is too small, the Hb molecules will be too closely packed to provide plasticity, whereas if the cell volume is too large, the Hb molecules will be so far apart that plasticity will be lost. We may accordingly accept plasticity as a function of cell volume and of the concentration of surplus Hb fragmentation, which depends on plasticity, being a function of tonicity, which regulates volume. The fragmentation-tonicity relation should, on this argument, show a maximum value, i.e. there should be one tonicity which is optimal for fragmentation. It is too difficult to investigate the fragmentation-tonicity relation for ghosts because fragments of ghosts cannot be readily enumerated, but the form of the relation, as well as that of other similar relations which present maxima or minima at tonicities which are optimal for a phenomenon, can be easily determined in systems which contain intact red cell.

VI. SOME PHENOMENA WHICH OCCUR MAXIMALLY AT OPTIMAL VALUES OF TONICITY

The two phenomena in which the dependence on tonicity shows the clearest maxima are the heat fragility of red cells and their resistance to mechanical haemolysis.

(1) *Heat fragility of red cells.* The washed red cells of fresh human blood are suspended in 1 % NaCl in a volume concentration of 0·4. One volume of this suspension is added to 4 vol. of 3, 2, 1·5, 1·0, 0·8 and 0·6 % NaCl; this gives systems whose tonicity T is approximately 2·75, 1·87, 1·43, 1·00, 0·83 and 0·65. After standing for 30 min., samples of each system are heated to a known temperature (between 52 and 56° C. in these experiments) for a known time (3 min. in these experiments). The number of cells N_0 in the systems before heating is found by counting; after the heating, the number N of cells plus fragments is counted. The fractional amount of haemolysis, p, in each system is found photometrically; the fragmentation is then measured by

$$f=\frac{N/N_0}{1-p},$$

haemolysis in these systems being substantially all-or-none (see Ponder, 1950*a*).

The results of an experiment of this type are shown in Table 2 and in Fig. 1.

Table 2

T	52° C., 3 min.		55° C., 3 min.		52° C., 3 min. plasma present	
	f	p	f	p	f	p
2·75	1·2	0·08	1·3	0·13	1·1	0·13
1·87	1·9	0·10	2·2	0·13	2·0	0·06
1·43	2·3	0·11	3·1	0·26	2·2	0·03
1·00	2·0	0·12	2·9	0·37	2·0	0·02
0·83	1·7	0·15	2·6	0·40	1·6	0·01
0·65	1·3	0·17	2·2	0·42	1·4	0·01

The value for the fragmentation f is at a maximum in a tonicity of about 1·5, becoming small at high and low tonicities. The value of p increases with decreasing tonicity. The maximum at $T = 1{\cdot}5$ is also observed in systems containing plasma (unwashed red cells being used in place of washed red cells). In a tonicity of 1·5, the volume of the human red cell is about 20 % smaller than it is in a tonicity of 1·0, and the Hb is accordingly about 1·25 times as concentrated as in the normal red cell. This appears to be the concentration (about 37 g. %) at which the viscous

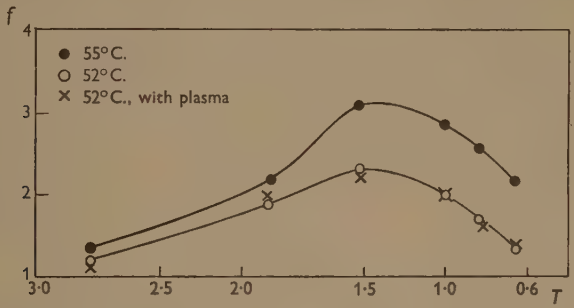

Fig. 1. Effect of heating human red cells in systems of various tonicities. The mean number of fragments, f, per red cell is plotted against tonicity T.

and plastic properties of the constituents of the red cell are optimal for the production of stable fragments. It is understandable that there should be an optimal value of T which provides a state intermediate between that in hypotonic media, with low Hb concentration and low cohesion, and that in hypertonic media, with Hb so concentrated as to approach paracrystalline or even crystalline arrangements.

(2) *Mechanical fragility of red cells.* The washed red cells of fresh human blood are suspended in 1 % NaCl in a volume concentration of 0·4, and are added to solutions of NaCl of various tonicity as described in the foregoing section but in the proportion of 1 ml. of suspension to 8 ml. of each NaCl solution. After standing for 30 min., the tubes containing the systems are centrifuged gently, and 8 g. of the supernatant fluid are removed into a weighed vessel. The 1 ml. (approximately) which remains contains the red cells in volume concentrations which are a little smaller, or a little greater, than 0·4, according to whether the red cell volume has decreased or increased as a result of the tonicity change.* The red cells in each tube are gently resuspended in the medium surrounding them,† and 0·5 ml. of each suspension is

* It is usual to measure mechanical fragility in systems of the same volume concentration, since the fragility tends to increase with increasing volume concentration. In these systems, the volume concentration in the hypertonic systems is less than that in the isotonic and hypotonic systems; if all the volume concentrations were adjusted so as to be equal, the mechanical fragility in the hypertonic systems would be increased, i.e. the minimum would become even more pronounced than it is.

† A little haemolysis is apt to occur during this resuspension, especially in hypertonic systems. In these experiments, this small amount of haemolysis has been measured and allowed for; this entails an additional and obvious technical step not described in detail.

transferred to the flasks of the mechanical fragility apparatus.* Each flask contains three glass beads. The flasks are rotated for 45 min. at a rate of 30 rev./min., at the end of which time the suspension in each is transferred to small tubes and centrifuged. The Hb in each supernatant fluid is determined photometrically, and expressed as a fraction of the total Hb of the system.

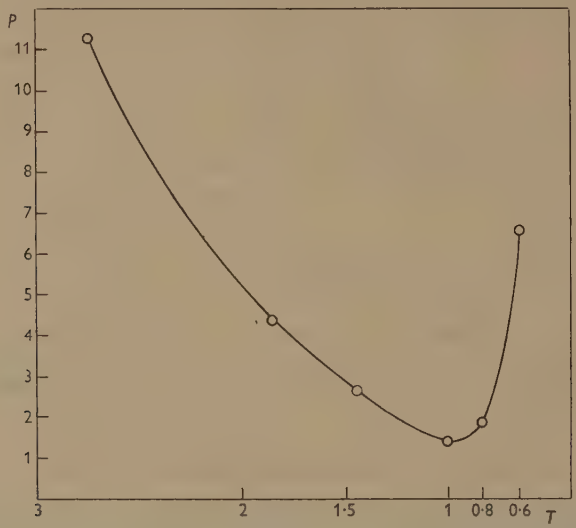

Fig. 2. Mechanical fragility of human red cells as a function of tonicity T. P is the percentage haemolysis produced in the system.

The results of an experiment of this kind are shown in Fig. 2. The mechanical fragility of the cells, as measured by the fractional haemolysis p, is at a minimum in a tonicity of 1·0, increasing as the tonicity is made greater or smaller. This suggests that when the cell has the volume corresponding to $T = 1{\cdot}0$ ($86\,\mu^3$), the cohesion of its constituents is such as to present a maximum resistance to the somewhat obscure forces to which it is exposed in the mechanical fragility apparatus. These forces must be of quite a different nature from those involved in heat fragmentation, a phenomenon which is at a maximum in about the same tonicity as that at which mechanical fragility is a minimum. It is known, in this connexion, that the lysis produced mechanically is all-or-none, and that it does not involve fragmentation. It has been suggested that a swollen red cell is less able to be distorted mechanically without its surface being stretched than a normally shaped red cell is, and that this may be the explanation for the increased mechanical

* Made by Mr Paul Cutajar of the New York University machine shop. It resembles Castle's mechanical fragility apparatus, but has the improvement that the constant speed of revolution can be varied through a ten-fold range.

fragility in hypotonic media; the observation that the fragility-tonicity relation has a minimum, however, suggests instead that the resistance to mechanical stresses depends on properties such as plasticity and the special cohesions which exist between the molecules of an ordered structure.

(3) *Remarks concerning other phenomena.* It is understandable that some of the properties inherent in a structure which is built in some special manner will be at a maximum or a minimum when the structure is neither compressed nor expanded, and it is interesting to examine a number of phenomena to see which of them are functions of tonicity, and which of them present maxima or minima. Phenomena which show maxima and minima may be suspected of being related to an optimal arrangement or separation of the molecules of the red cell ultrastructure, and if we distinguish somewhat arbitrarily between the surface ultrastructure, with dimensions of l^2, and the ultrastructure of the interior, with dimensions of l^3, the phenomena in question will be more likely related to the latter than to the former. The phenomena of heat fragmentation and mechanical fragility, which may obviously involve properties of plasticity and cohesiveness (l^3 properties) are good examples of this idea.

Looked at in this way, it is not surprising that disk-sphere transformations, whether produced by distearyl lecithin or sodium tetradecyl sulphate, are found to have no tonicity dependence when examined quantitatively by methods which have already been described (Ponder, 1947). The disk-sickle shape transformation, on the other hand, is greatly affected by tonicity. The typical sickle form occurs only at a tonicity in the neighbourhood of 1·0; in hypotonic media, it is replaced by a large atypical sickle with rounded outlines and little filament formation, while in hypertonic media ($T=3$) the typical sickles are replaced by 'holly wreath' forms with multiple foci of distortion. These results are understandable in terms of the sickling shape change having a dependence on l^3 and accordingly showing a tonicity maximum, whereas the disk-sphere transformations have a dependence on l^2 and have no maximum or minimum in their tonicity dependence; indeed, they do not seem to have any tonicity dependence at all.

Two other phenomena which have a well-marked maximum in their tonicity dependence have been found in the course of a search for phenomena of this type. The first occurs in systems containing human red cells and the lysins sodium dodecyl sulphate or sodium tetradecyl sulphate; the velocity of haemolysis by these substances, in concentrations which approach their asymptotic concentrations, is at a maximum in tonicities between 1·0 and 1·5, and becomes much less in more hypertonic or hypotonic systems. There are many examples of lysins which have lytic effects with a tonicity dependence (e.g. the lytic effect of saponin decreases with decreasing tonicity and the lytic effect of lysolecithin increases with decreasing tonicity (Ponder, 1937; Wilbur & Collier, 1943); the maximum found in systems containing the detergents is very unusual. The most unexpected minimum in a tonicity dependence, however, is that in the K-Na exchange which occurs in human red cells kept for about 24 hr. at 4° C. This exchange is at a minimum at a tonicity of about 0·7; in a tonicity of 3, it is almost double its minimum value,

and at a tonicity of 0·5 it is slightly greater than the minimum value.* The presence of the minimum suggests that the exchange has a dependence of the I^3 type rather than one of the I^2 type, and could be used as an additional indirect argument against its being a simple diffusion phenomenon taking place along concentration gradients.

SUMMARY

1. A large number of kinds of ghost, each with its own characteristic properties, can be prepared from human red cells. These ghosts, which differ from each other in their appearance as seen with phase optics, in their heat fragmentation, and probably in other respects also, are the result of the procedures used to prepare them (lysis by water as opposed to lysis in dilute saline, lysis followed by 'reversal of haemolysis' by phosphate or veronal buffers at various pH or by saturating the haemolysate with CO_2, lysis by freezing and thawing, lysis by chemical lysins, etc.). In view of this, it is meaningless to speak of the 'red cell ghost' and of its structure without further specification.

2. No entirely satisfactory explanation for the differences in heat fragmentation of the various kinds of ghost has been found. The best one is that fragmentation into stable fragments occurs when the ghost is discoidal and when it has a certain plasticity, the plasticity depending on the concentration of residual Hb. It is shown that heat fragmentation of red cells occurs maximally in a tonicity of about 1·5, i.e. when the Hb molecules are a certain optimal distance apart.

3. A number of other phenomena which occur optimally at certain values of tonicity, i.e. when the Hb molecules are neither too closely packed nor too widely separated, are described. Among these phenomena are mechanical fragility, the formation of typical sickles, resistance to certain lysins, and K-Na exchange. The various forms of disk-sphere transformation seem to have no tonicity dependence.

REFERENCES

DAVSON, H. (1937). The loss of potassium from the erythrocyte in hypotonic saline. *J. Cell. Comp. Physiol.* **10**, 247–64.

DERVICHIAN, D. G. & MAGNANT, C. (1947). Contribution à l'étude du mechanisme de l'hemolyse. *Ann. Inst. Pasteur*, **73**, 841–50.

FURCHGOTT, R. F. (1940). Observations on red cell ghosts. *Cold Spr. Harb. Symp. Quant. Biol.* **8**, 224–32.

FURCHGOTT, R. F. & PONDER, E. (1941). Electrophoretic studies on human red blood cells. *J. gen. Physiol.* **24**, 447–57.

MICHAELIS, L. (1931). Der Acetat-Veronal-Puffer. *Biochem. Z.* **234**, 139.

PARPART, A. K. (1942). The preparation of red cell membranes. *J. Cell. Comp. Physiol.* **19**, 248–51.

PONDER, E. (1937). Effects of simple haemolysins in hypotonic systems. *Protoplasma*, **27**, 523–9.

PONDER, E. (1942). On properties of the red cell ghost. *J. Exp. Biol.* **18**, 257–65.

* The increased ion exchange in hypotonic systems does not appear until tonicities are reached which are so low as to be nearly haemolytic (e.g. $T = 0{\cdot}5$). Davson (1937) described this effect of hypotonicity, which I was unable to reproduce at tonicities in the neighbourhood of 0·6 (Ponder, 1949). Davson's explanation for the effect, which involved the conception of the cell membrane becoming permeable when stretched, cannot account for the fact that the relation between ion exchange and tonicity has a minimum value.

Ponder, E. (1947). Disk-sphere transformations produced by lysins, and their reversal, in systems of different pH. *J. Exp. Biol.* **23**, 350–6.

Ponder, E. (1949). The rate of loss of potassium from human red cells to which lysins have not been added. *J. Gen. Physiol.* **32**, 461–79.

Ponder, E. (1950*a*). A quantitative study of the fragmentation and haemolysis of mammalian red cells by heat. *J. Exp. Biol.* **27**, 198–209.

Ponder, E. (1950*b*). Observations sur certaines propriétés des stromas de globules rouges. *Revue Hemat.* **5**, 580–90.

Ponder, E. (1951). Fragmentation of red cell ghosts in relation to the problem of red cell structure. *J. Exp. Biol.* **28**, 567–75.

Wilbur, K. M. & Collier, H. B. (1943). A comparison of the hemolytic actions of lysolecithin and saponin. *J. Cell. Comp. Physiol.* **22**, 233–49.

HORMONE BALANCE AND THE CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS* (HEMIPTERA)

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(With Plates 22 and 23 and Two Text-figures)

The body cells of the developing insect carry the potentialities for both larval and imaginal differentiation. During larval life imaginal differentiation is suppressed because in the presence of the juvenile hormone (sometimes referred to as the 'inhibitory hormone'), secreted by the corpus allatum, the intracellular system which leads to the formation of larval structures takes precedence over the system which leads to the formation of adult structures (Wigglesworth, 1940).

If the corpus allatum in *Rhodnius* is removed by decapitation at varying times in the moulting cycle of a young larva, when varying amounts of juvenile hormone have been secreted, all intermediate grades of metamorphosis between larva and adult can be produced (Wigglesworth, 1934). In the normal insect there is a nicely adjusted balance between the juvenile hormone and the moulting hormone;* in these experiments the balance has been grossly upset. The operation of this hormone balance has been well illustrated by the work of Piepho (1940, 1950*a*) on *Galleria*.

In the course of normal development in *Rhodnius* there is a small amount of differentiation towards the adult form in each larval instar. This is most striking at the moult from the 4th to the 5th instar, where the differentiation of the external genitalia, the enlargement of the wing pads, the sharp fall in the rate of increase in the number of abdominal plaques and bristles (Wigglesworth, 1940) are all indications of limited imaginal differentiation.

These fine adjustments during growth are also the result of controlled hormone balance. In the present paper some of the ways in which this balance may be upset are described—with the object of throwing light upon the mechanisms by which the correct balance is normally maintained.

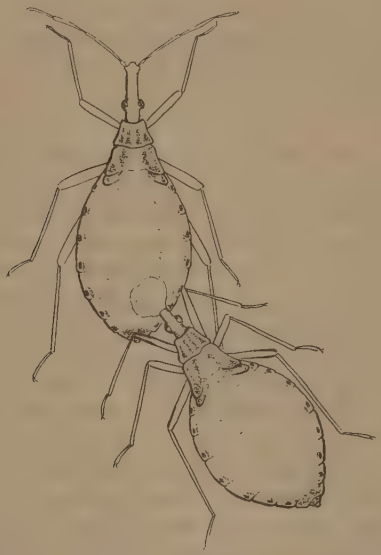
(i) *Transfusion from an earlier larval stage*

It has been shown already that if the 4th-stage larva of *Rhodnius* is decapitated 1 day after feeding and joined to a 3rd-stage larva, retaining its corpus allatum, which has just passed the critical period, the 4th-stage larva does not develop the

* As pointed out elsewhere (Wigglesworth, 1952), the 'moulting hormone' is composite and consists of a factor secreted in the brain that activates the thoracic gland which then secretes the hormone which initiates growth and moulting.

characters of a 5th stage, but develops again those of the 4th. It was from this experiment that it was inferred that 'the characters of the various instars are controlled by the corpus allatum in the same way as metamorphosis is controlled' (Wigglesworth, 1936).

In these experiments the insects were joined neck to neck and were therefore unable to escape from the cuticle at moulting. Consequently, it was not possible to detect small differences in the differentiation of the crumpled wing lobes; all the conclusions were based on the minute growth changes in the rudiments of the external genitalia.



Text-fig. 1. 4th-stage larva of *Rhodnius* with a second 4th-stage larva joined to the abdomen.

A new technique has now been adopted in which a small hole is cut in the abdomen of the insect that is to be subject to experiment; the head of the insect that is to be used for transfusion, after cutting off the tip, is inserted into this hole and the margin sealed with paraffin wax (Text-fig. 1). It is advisable also to secure the legs of the second insect with paraffin. Under these conditions the first insect is often able to free the head and thorax from the old cuticle at moulting and to expand the wing lobes, etc., in a normal manner.

Pl. 22, fig. 1, shows the normal 4th-stage larva and Pl. 22, fig. 2, the normal 5th stage. Pl. 22, fig. 3, shows a larva produced from a 4th stage to which had been joined by the above technique, at 1 day after feeding, a 3rd-stage larva at 5 days after feeding. In this experiment the two insects moulted simultaneously 14 days after the union. The 4th-stage larva has developed into a 5th stage with wing lobes which have differentiated little more than in the normal 4th stage.

It seems probable from this experiment that the corpus allatum secretes into

the blood a higher concentration of juvenile hormone during the 3rd stage than during the 4th. It might be argued that this results from a simple relationship between the relative volumes of the gland and the total body fluid (cf. Novak, 1951). But if the 4th-stage larva in the above experiment is decapitated, it produces characters (in the genitalia and the crumpled wing lobes) which show just as little change towards those of the 5th instar as does the 4th-stage larva which retains its corpus allatum. Thus a single 3rd-stage corpus allatum acting upon combined larvae of 3rd and 4th stages is just as effective as the combined corpora allata of the two larvae.

One must conclude that the corpus allatum of the 3rd-stage larva is adapted to raise and maintain the concentration of juvenile hormone in the blood at a level characteristic of that instar. But there is another factor to be considered: the timing of the secretion.

(ii) *Transfusion from the same larval stage at different periods in the moulting process*

There is evidence that in the normal process of moulting the juvenile hormone is not secreted into the blood until after the critical period (Wigglesworth, 1934). The time of exposure of the tissues to this hormone has been varied experimentally by transfusing 4th-stage larvae with the blood of other larvae, also in the 4th stage, which had been fed some days earlier. The same technique was used: batches of 4th-stage larvae at 24 hr. after feeding had connected to them, towards the hind end of the abdomen, other 4th-stage larvae fed 2, 3, 4, 6, 7 and 8 days previously.

Those joined to 2- and 3-day larvae gave rise to normal 5th-stage larvae (Pl. 22, fig. 4). Of those joined to 4-day larvae, one (out of six) developed genital rudiments which had not differentiated quite so much as they should. Of those joined to the 6-day larvae, all gave rise to slightly juvenile forms in which the wing lobes did not extend quite so far backwards as in the normal 5th stage.

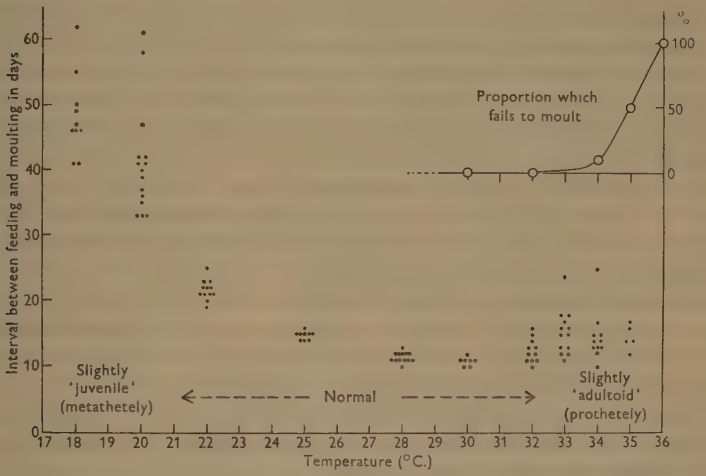
In those joined to larvae fed 7 and 8 days earlier, the juvenile characters were more striking. Pl. 22, figs. 5 and 6, shows two of these larvae: the wing lobes are not so juvenile in form as those produced by transfusion from a 3rd-stage larva (cf. Pl. 22, fig. 3), but they are far smaller than those of the normal 5th-stage larva (cf. Pl. 22, fig. 4). The genitalia show corresponding changes.

In these experiments there can be no question of the juvenile hormone being present in higher concentration than usual. But it has been introduced into the system too early in the moulting process. Instead of being first produced in notable quantities when growth has already been initiated by the 'moulting hormone', it is present in the blood from the commencement of the moulting process.

(iii) *Effect of abnormal temperatures*

Batches of 4th-stage larvae of *Rhodnius* were placed, immediately after feeding, in a humid atmosphere at temperatures ranging from 18 to 37° C. Text-fig. 2 shows the number of days after feeding at which moulting took place. At 28–30° C. moulting requires about 11 days. Below this temperature moulting is progressively retarded and the individual variation becomes very great. At 18° C. the time

required varies from 40 to 60 days. Above 30° C., also, moulting is slightly retarded and there is a large amount of scatter. At 34° C. some larvae (about 10 %) fail to moult altogether; at 35° C. about 50 % fail to moult; and at 36° C., and above none moults.* If they are fed again and placed at the optimum temperature these larvae moult normally.



Text-fig. 2. Effect of temperature on the moulting of 4th-stage larvae of *Rhodnius*.

There are no very striking morphological differences in the 5th-stage larvae produced at these different temperatures; those at the higher temperatures are slightly paler, but all have unmistakable 5th-instar characters. When examined closely, however, slight differences are detectable. Some of the females moulting at the lowest temperatures have the genital lobes slightly less differentiated than normally; and of the females moulting at the higher temperatures an increasing proportion shows the first pair of valvulae slightly separated (as shown in Wigglesworth, 1948, text-fig. 2B). The numbers of females concerned were very small but the proportion showing this change was as follows: 32° C., 50 %; 33° C., 60 %; 34° C., 85 %; 35° C., 100 %. This slight separation is an 'adultoid' character.

A better character for detecting these very slight changes is afforded by the anterior wing lobes. Pl. 23, figs. 1, 3 and 5, shows the form of these in 5th-stage larvae that had moulted at 18, 25 and 34° C. At the low temperature (Pl. 23, fig. 1) the distal half of the wing (the 'membrane') is much less clearly marked off from the basal half (the 'corium'). At the high temperature (Pl. 23, fig. 5) the 'membrane' is relatively smooth and is thus sharply differentiated from the 'corium' which is roughened by prominent plaques.

* The lethal temperature, defined here as the temperature at which 50 % of larvae 1 day after feeding are killed within 24 hr. is about 40° C.

Microscopically the difference is well seen in the type of cuticle that occurs in the proximal region of the 'membrane' (Pl. 23, figs. 2, 4 and 6). In the normal 5th-stage larva there are well-developed plaques bearing bristles in the basal half of the wing pad. In the distal half many of the plaques and bristles have disappeared, although the sites at which they occurred in the previous instar can still be seen in the sculpturing of the surface (Pl. 23, fig. 4). In 5th-stage larvae produced at the lowest temperatures, many more plaques have persisted and the bristles which they bear are larger than in the normal 5th stage (Pl. 23, fig. 2). In 5th-stage larvae produced at the highest temperatures, the reduction of plaques has proceeded further than in the normal insect, and the wing lobes, particularly the distal parts, are relatively smooth, and the few remaining bristles much reduced in size (Pl. 23, fig. 6).

At low temperatures the wing lobes are clearly more 'juvenile' in character than those of the normal 5th-stage larva, at high temperatures they are more 'adultoid'. Those at the low temperatures may be described as exhibiting 'metathetely', those at the high temperatures exhibit 'prothetely'.

Clearly, low temperature upsets the hormone balance very slightly in favour of the juvenile hormone, while the high temperature upsets the balance very slightly in favour of the 'moulting hormone'. But as the temperature rises above 35–36° C. the secretion of the moulting hormone fails, although the other metabolic processes in the insect are not visibly affected. Whether it is the 'activating' component from the brain, or the 'moulting' factor from the thoracic gland (Wigglesworth, 1952) which is lacking has not been determined.

(iv) *Effect of deficient oxygen*

Groups of four 4th-stage larvae, 1 day after feeding, were placed at 25° C. in 250 c.c. conical flasks containing different concentrations of oxygen. The gas mixture was changed each day.

In 99 % oxygen the larvae were all dead within 5 days. In 75 and 50 % oxygen normal 5th-stage larvae were produced. In 5 % oxygen moulting was delayed; it required 19–32 days compared with the normal 14–16 days in air. The resulting 5th-stage larvae, as judged by the genitalia and the plaques on the wing lobes, were very slightly 'adultoid'; that is, like the larvae exposed to high temperature they show very slight prothetely. In 2·5 % oxygen all died within 11 days without any sign of moulting beginning.

(v) *Implantation of the corpus allatum of* Rhodnius *and* Periplaneta

We have seen that 4th-stage larvae transfused with blood from intact 3rd-stage larvae develop 4th-instar characters again when they moult. But the implantation of corpora allata from 3rd-instar larvae, at a week or so after feeding, into 4th-stage larvae 1 day after feeding, does not have that effect: perfectly normal 5th-instar larvae are produced. Presumably the interference with the tracheal supply of the implanted gland causes a temporary suppression of hormone secretion; or, as

suggested previously (Wigglesworth, 1948), the corpus allatum of the host may control the level of concentration of the juvenile hormone.

The corpus allatum of the adult insect again secretes juvenile hormone which is necessary for yolk production (Wigglesworth, 1948). It was shown by Novak (1951) that the corpus allatum of the adult *Periplaneta* will cause the development of larval characters at moulting in the Lygaeid *Oncopeltus*. This has been confirmed in *Rhodnius*: of ten 5th-stage larvae, each of which received implants of a pair of corpora allata from adult *Periplaneta*, all gave rise to 6th-stage larvae or intermediates; of those which moulted again, six gave rise to 7th-stage larvae and one to a 7th-stage adult. The gland therefore continues to secrete juvenile hormone for a long time after implantation. In histological sections the implanted corpus allatum of *Periplaneta* appears normal and healthy. (The apparent identity of the juvenile hormone in both hemimetabolous and holometabolous insects has been described by Piepho (1950*b*).)

Corpora allata from mature adult *Periplaneta* were implanted into 4th-stage larvae of *Rhodnius* 24 hr. after feeding; but, as in the case of larvae receiving implants of corpora allata from 3rd-stage larvae of *Rhodnius*, they developed normal 5th-instar characters.

(vi) *Change in function of the corpus allatum in the last larval stage*

Metamorphosis results from the failure of the corpus allatum in the last larval stage to secrete the juvenile hormone. In order to see whether the gland plays a more active part in bringing about metamorphosis, corpora allata from 5th-stage larvae or from newly moulted adults were implanted into 2nd-, 3rd- and 4th-stage larvae. In many of these there was a partial metamorphosis at the moulting of the 4th stage (Wigglesworth, 1948).

This result signifies that the implanted corpus allatum is acting upon the corpus allatum of the host and weakening its capacity to secrete the juvenile hormone, or that it is actively removing juvenile hormone from the blood, or that it is now secreting a hypothetical 'metamorphosis hormone' which favours the differentiation of adult characters.

In the earlier paper the explanation preferred was an active removal of juvenile hormone. The grounds for this preference were that if the 5th-stage larva of *Rhodnius* had the brain removed and was then caused to moult by joining it to another 5th-stage larva which had just passed the critical period, then the imaginal differentiation, particularly of the wing lobes, was more complete when the insects retained their corpora allata than when these were removed. It was therefore inferred that in the normal 5th-stage larva some juvenile hormone is still present in the blood and that it is removed by the altered activity of the corpus allatum during the final moult.

These experiments have been repeated in modified form. A number of 4th-stage and 5th-stage larvae were fed within 2–5 days after moulting (so that they might be expected to have the maximum amount of juvenile hormone persisting). They were decapitated at 24 hr. after feeding and caused to moult by implanting

into the abdomen the thoracic glands from 5th-stage larvae at 10 days after feeding (Wigglesworth, 1952). Half of the decapitated larvae received, in addition, an implant of the corpus allatum from the 5th-stage larvae.

In this way nineteen 4th-stage larvae moulted without the corpus allatum, twenty moulted with the corpus allatum of a 5th-stage larva. All developed adult characters; and there was no detectable difference in the differentiation of the wings, abdominal cuticle or genitalia between the two groups.

Among the 5th-stage larvae, eleven without the corpus allatum moulted and nine with the corpus allatum. All developed adult characters. In many of them the folding of the wing lobes was deficient, and in some the wings were like those shown in Wigglesworth (1948, pl. 1, fig. 2). But these deficiencies occurred with equal frequency in the two groups. They appear to be the result of decapitation, which may perhaps interfere with the circulation of blood in the wing lobes.

These results, therefore, give no indication of any notable persistence of juvenile hormone from one instar to the next, and they invalidate the evidence on which it was concluded that there is an active removal of juvenile hormone by the corpus allatum at the final moult. Such an active removal may still be the explanation of the results recalled at the beginning of this section; but it is equally possible that the hormone balance is being slightly upset in some other way. It is worth recalling that Pflugfelder (1939) observed that the implantation of corpora allata into *Dixippus* leads to a partial suppression of growth in the corpora allata of the host insect.

DISCUSSION

Prothetely and metathetely

The new experiments described and the old experiments recalled in this paper illustrate the various ways in which the hormone balance may be upset and abnormalities in metamorphosis produced. In the unoperated insect such abnormalities take the form of prothetely, metathetely or neoteny. These abnormal forms were quoted by Goldschmidt (1923) as examples of his principle of the control of morphological characters by differential reaction velocities; they were regarded as the result of an upset in the time relations of two processes going forward simultaneously.

According to Goldschmidt the two processes were the 'evagination of wing buds' and 'metamorphosis'. This same explanation was adopted by v. Lengerken (1924*a*, *b*) who considered one of the processes in question to be the formation of the oxidase which Dewitz (1902) had supposed necessary for metamorphosis: deficient production of this enzyme resulting in delayed or incomplete metamorphosis (metathetely), excessive production leading to prothetely.

The recognition of the control of metamorphosis by the balanced action of two hormones provided a more precise description of prothetely and metathetely (Wigglesworth, 1934; Piepho, 1942). In a later paper it was suggested that the two competing processes were (i) 'differentiation towards the adult form' brought about by the moulting hormone, and (ii) the deposition of the new cuticle brought about by the juvenile or inhibitory hormone. If the second process was accelerated

metathetely or partial restraint of metamorphosis was the result; if this process was delayed precocious metamorphosis or prothetely supervened (Wigglesworth, 1936).

But a comparative study of the epidermis during larval moulting and during the metamorphic moult showed that this explanation is too crude: the process of growth is different from the outset if the juvenile hormone is present. Moreover, if the adult is caused to moult in the presence of the juvenile hormone it suffers a partial *reversal* of metamorphosis, which cannot be explained by an early deposition of cuticle. The description was therefore elaborated by supposing that the intracellular system which is responsible for the formation of larval characters, and which is activated by the juvenile hormone in the presence of the moulting hormone, works more rapidly than the system responsible for the formation of adult characters. It is for this reason, it was suggested, that larval characters predominate in the presence of the juvenile hormone (Wigglesworth, 1940).

But, as was pointed out by Pfeiffer (1945), and as has become apparent in the course of further experiments on *Rhodnius*, the length of time required for moulting shows no constant relation to the characters, larval or adult, that are eventually produced. The characters produced are determined by the balance between two opposing hormone formulae: moulting hormone alone, and moulting hormone plus a variable amount of juvenile hormone. The final result is controlled by the relative concentration of the juvenile hormone and by the stage in moulting at which it is introduced into the system. The parallel with sex determination and intersex formation has been pointed out before.

The best-known environmental agencies which may bring about abnormalities of metamorphosis are temperature and asphyxiation. As long ago as 1813 Majoli (quoted by Pruthi, 1924) observed prothetely in silkworms under the influence of high temperature. Pruthi (1924) obtained *Tenebrio* larvae with wing rudiments on exposure to high temperature (29·5° C.) and Arendsen-Hein (quoted by v. Lengerken, 1932) observed metathetely in the same insect after transfer to abnormally cold conditions. Nagel (1934) obtained the same result in *Tribolium*. Other examples are quoted by Thomas (1932). (Cf. Radtke, 1942.)

There are fewer observations on the effect of asphyxiation, but Dewitz (1902) obtained intermediates between larvae and pupae of *Pieris* when the larvae had been placed in sealed tubes.

In the present work high temperature is shown slightly to depress the action of the juvenile hormone in *Rhodnius* so that the resulting insect shows a mild degree of prothetely; low temperature slightly enhances the action of the juvenile hormone so that a mild degree of metathetely results. A low partial pressure of oxygen acts like high temperature and slightly depresses the action of the juvenile hormone. The experiments do not reveal the site of action of these environmental factors.

Cause of metamorphosis

There is much that is still obscure about the causation of metamorphosis. It is clear that the absence of the juvenile hormone at the final larval moult leaves the larval system unactivated so that the adult system may differentiate and meta-

morphosis occur. But there is much uncertainty as to the part played by the developing tissues themselves—that is, by their responsiveness or competence to differentiate.

The 1st-stage larva of *Rhodnius* deprived of its corpus allatum and transfused with moulting hormone from the 5th-stage larva undergoes metamorphosis (Wigglesworth, 1934). Larvae of Lepidoptera in the 2nd instar transform into diminutive pupae and moths if the corpora allata are removed (Bounhiol, 1938); and isolated fragments of integument from caterpillars, even in the 1st instar newly hatched from the egg, can be induced to pupate (Piepho, 1938).

In these insects the tissues are clearly capable of metamorphosis at any stage of larval growth. But there are some insects in which this is less certain. If the corpora allata are removed from larvae of *Dixippus* in the 3rd stage they make two more moults before they begin to lay eggs (Pflugfelder, 1937). Similarly, the cockroach *Leucophaea* (which has an average of eight larval instars) deprived of its corpora allata in the 5th stage, first moults to give rise to an intermediate 'pre-adultoid form' and then moults a second time to produce a diminutive adult (Scharrer, 1946).

Scharrer (1946, 1948) attributes this delay in the appearance of adult characters to the tissues being as yet incapable of metamorphosis. That certainly appears to be the case in the experiments of Bodenstein on isolated imaginal disks from *Drosophila*: salivary glands transplanted into mature larvae will not undergo metamorphosis unless they have reached a fairly advanced stage of growth (Bodenstein, 1943*a*); and the other organ disks vary in their responsiveness to the ring gland hormone in a definite order (Bodenstein, 1943*b*). 'Whether the organ disks respond...with growth or differentiation depends on a definite relation between hormone concentration and organ responsiveness' (Bodenstein, 1943*b*).

It may well be that in the higher Diptera in which the germs of the imaginal organs are completely independent of the larval integument, these imaginal disks must reach an advanced stage of growth before they can undergo differentiation. But in view of the ready metamorphosis of Hemiptera and Lepidoptera at a very early stage of larval growth it is unlikely that the delay in metamorphosis after removal of the corpus allatum in the Orthoptera *Dixippus* (Pflugfelder, 1937) and *Leucophaea* (Scharrer, 1946) is due to a lack of responsiveness in the tissues. All these are insects in which the imaginal potencies are latent in cells which play a functional part in the larval organism.

It was on these grounds that it was suggested (Wigglesworth, 1948) that this delay might be due to the persistence of juvenile hormone in the blood or tissues from one instar to the next. And it was further suggested that the active removal of such persistent juvenile hormone might be a necessary step in the process of metamorphosis.

In the present work no evidence has been obtained for the persistence of any juvenile hormone in *Rhodnius*, and it has therefore not been possible to test the hypothesis that the corpus allatum, when it ceases to secrete the juvenile hormone

at the last moult, also actively removes or inactivates the traces of that hormone persisting in the blood. But this hypothesis is still worthy of being tested on other insects. In *Rhodnius* the removal of the corpus allatum by decapitation has been carried out before any juvenile hormone has been secreted; but it may well be that at the time of this removal in *Dixippus* (Pflugfelder, 1937) and *Leucophaea* (Scharrer, 1946) the blood already contains some juvenile hormone. In that case it is not surprising that the ensuing moult should give rise to a larval form. In the silkworm the corpus allatum in the last or 5th-stage larva appears to change its function during the instar: early in the instar it secretes juvenile hormone, but after the fourth day it no longer does so (Fukuda, 1944).

There remains the problem of what causes the corpus allatum to change its function at the last larval moult so as to allow metamorphosis to occur. It has been found again in the course of the present work (cf. Wigglesworth, 1948) that the isolated corpus allatum implanted into the abdomen no longer regulates its function in this way. One must conclude that the normal regulation is dependent on its connexion with the central nervous system and that the signal to the corpus allatum to change its secretory activity comes from the brain.

SUMMARY

A technique is described by which the intact larva of *Rhodnius* can be transfused with blood from another larva without interfering with ecdysis.

If the 4th-stage larva receives blood from a 3rd-stage larva it develops characters little different from those of the 4th instar. This is attributed to the 3rd-stage larva producing juvenile hormone at a higher concentration.

If the 4th-stage larva at 24 hr. after feeding receives blood from another 4th-stage larva at 8 days after feeding it develops characters intermediate between those of the 4th and 5th instars. This is attributed to the juvenile hormone being introduced too early in the moulting cycle.

The hormone balance is upset by abnormal temperatures. The 4th-stage larva will not moult at a temperature of 36° C. although the larvae can survive up to about 40° C.

At temperatures a little below 36° C. moulting is somewhat delayed and the characters developed are slightly 'adultoid' (prothetely). This is attributed to slightly reduced activity of the corpus allatum.

At temperatures below 20° C. moulting is greatly delayed and the characters developed are slightly 'juvenile' (metathetely). This is attributed to relatively increased activity of the corpus allatum.

Low concentrations of oxygen (less than 5 %) have an effect similar to that of high temperature.

If 5th-stage larvae of *Rhodnius* receive implants of corpora allata from mature adults of *Periplaneta* they develop into 6th-stage larvae and many of these subsequently into 7th-stage larvae. The 'juvenile hormone' appears to be the same in the two insects.

No evidence could be obtained for the persistence of juvenile hormone in the

blood from one instar of *Rhodnius* to the next. The hypothesis of an active elimination of juvenile hormone by the corpus allatum at the time of metamorphosis remains therefore unproven.

REFERENCES

BODENSTEIN, D. (1943*a*). Factors influencing growth and metamorphosis of the salivary gland in *Drosophila*. *Biol. Bull. Woods Hole*, **84**, 13–33.

BODENSTEIN, D. (1943*b*). Hormones and tissue competence in the development of *Drosophila*. *Biol. Bull. Woods Hole*, **84**, 35–58.

BOUNHIOL, J. (1938). Recherches expérimentales sur le déterminisme de la métamorphose chez les Lépidoptères. *Bull. Biol.* (Suppl.), **24**, 1–199.

DEWITZ, J. (1902). Untersuchungen über die Verwandlung der Insektenlarven. *Arch. Anat. Physiol., Lpz.* (*Abt. Physiol.*), pp. 327–40.

FUKUDA, S. (1944). The hormonal mechanism of larval moulting and metamorphosis in the silkworm. *J. Fac. Sci. Tokyo Univ.* Ser. IV, **6**, 477–532.

GOLDSCHMIDT, R. (1923). Einige Materialien zur Theorie der abgestimmten Reaktionsgeschwindigkeiten. *Arch. mikr. Anat.* **98**, 292–313.

v. LENGERKEN, H. (1924*a*). Prothetelie bei Coleopterenlarven. *Zool. Anz.* **58**, 179–85.

v. LENGERKEN, H. (1924*b*). Prothetelie bei Coleopterenlarven (Metathelie). *Zool. Anz.* **59**, 323–80.

v. LENGERKEN, H. (1932). Nachhinkende Entwicklung und ihre Folgeerscheinungen beim Mehlkäfer. *Jena. Z. Naturw.* **67**, 260–73.

NAGEL, R. H. (19 4). Metathetely in larvae of the confused flour beetle (*Tribolium confusum*, Duval). *Ann. Ent. Soc. Amer.* **27**, 425–8.

NOVAK, V. (1951). New aspects of the metamorphosis of insects. *Nature, Lond.*, **167**, 132–3.

PFEIFFER, I. W. (1945). The influence of the corpora allata over the development of nymphal characters in the grasshopper *Melanoplus differentialis*. *Trans. Conn. Acad. Arts Sci.* **36**, 489–515.

PFLUGFELDER, O. (1937). Bau, Entwicklung und Funktion der corpora allata und cardiaca von *Dixippus morosus* Br. *Z. wiss. Zool.* **149**, 477–512.

PFLUGFELDER, O. (1939). Wechselwirkungen von Drüsen innerer Sekretion bei *Dixippus morosus* Br. *Z. wiss. Zool.* **152**, 384–408.

PIEPHO, H. (1938). Über die experimentelle Auslösbarkeit überzähliger Häutungen und vorzeitiger Verpuppung an Hautstücken bei Kleinschmetterlingen. *Naturwissenschaften*, **26**, 841–2.

PIEPHO, H. (1940). Über die Hemmung der Verpuppung durch corpora allata. Untersuchungen an der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **60**, 367–93.

PIEPHO, H. (1942). Untersuchungen zur Entwicklungsphysiologie der Insektenmetamorphose. Über die Puppenhäutung der Wachsmotte *Galleria mellonella* L. *Roux Arch. Entw. Mech. Organ.* **141**, 500–83.

PIEPHO, H. (1950*a*). Über die Hemmung der Falterhäutung durch corpora allata. Untersuchungen an der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **69**, 261–71.

PIEPHO, H. (1950*b*). Über die Ausmass der Artunspezifität von Metamorphosehormonen bei Insekten. *Biol. Zbl.* **69**, 1–10.

PRUTHI, H. S. (1924). Studies in insect metamorphosis. I. Prothetely in mealworms (*Tenebrio molitor*) and other insects. *Proc. Camb. Phil. Soc.* (*Biol.*), **1**, 139–47.

RADTKE, A. (1942). Hemmung der Verpuppung beim Mehlkäfer *Tenebrio molitor* L. *Naturwissenschaften*, **30**, 451–2.

SCHARRER, B. (1946). The role of the corpora allata in the development of *Leucophaea maderae* (Orthoptera). *Endocrinology*, **38**, 35–45.

SCHARRER, B. (1948). Hormones in insects. *The Hormones*, ed. by S. Pincus and K. V. Thimann, **1**, 121–52.

THOMAS, C. A. (1932). Prothetely in an Elaterid larva (Coloptera). *Ent. News*, **44**, 91–6.

WIGGLESWORTH, V. B. (1934). Physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and metamorphosis. *Quart. J. Micr. Sci.* **76**, 269–318.

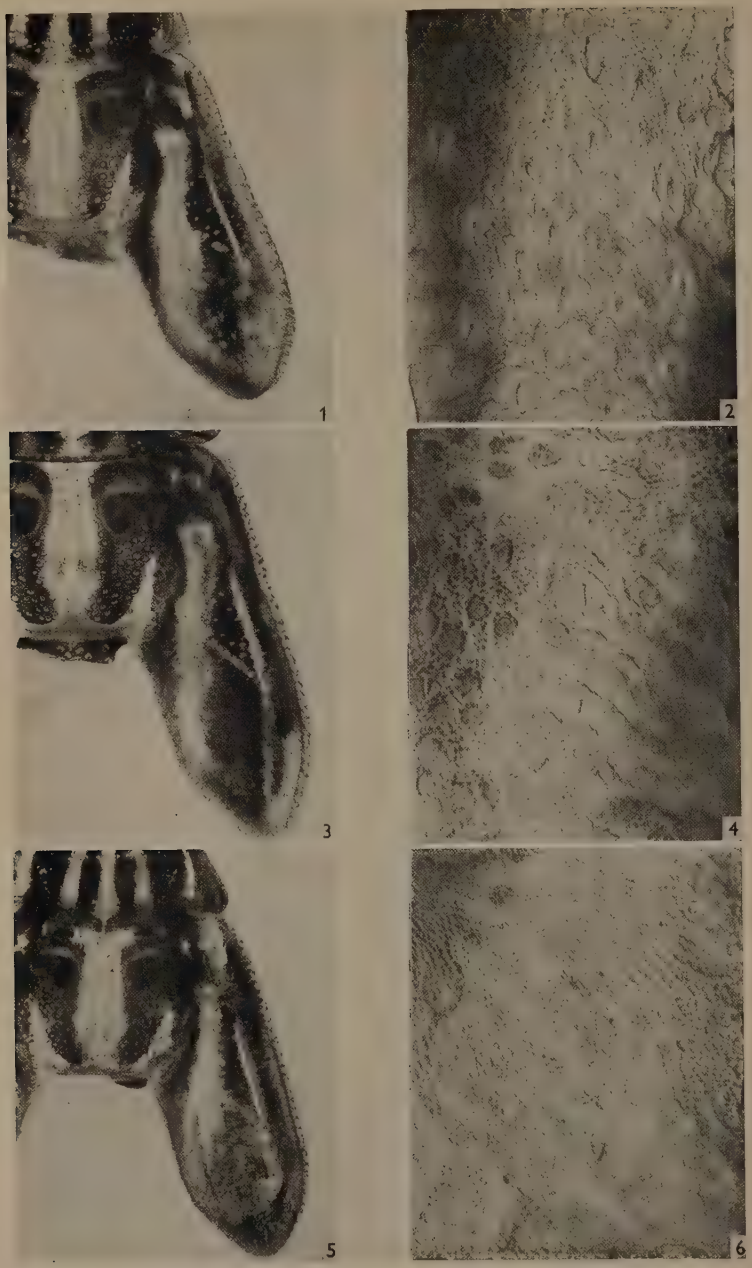
WIGGLESWORTH, V. B. (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. Micr. Sci.* **79**, 91–121.

WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17**, 201–22.

WIGGLESWORTH, V. B. (1948). The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **25**, 1–14.

WIGGLESWORTH, V. B. (1952). The thoracic gland in *Rhodnius prolixus* (Hemiptera) and its role in moulting. *J. Exp. Biol.* **29**, 561–70.

WIGGLESWORTH—CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS*



WIGGLESWORTH—CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS*

EXPLANATION OF PLATES

Plate 22

Fig. 1. Normal 4th-stage larva of *Rhodnius*.
Fig. 2. Normal 5th-stage larva.
Fig. 3. 5th-stage larva produced from 4th stage to which a 3rd-stage larva had been joined.
Fig. 4. 5th-stage larva produced from 4th stage to which another 4th-stage larva 2 days after feeding had been joined.
Fig. 5. As fig. 4, but second 4th-stage larva joined at 7 days after feeding.
Fig. 6. As fig. 4, but second 4th-stage larva joined at 8 days after feeding.

Plate 23

Fig. 1. Anterior wing lobe of 5th-stage larva produced at 18° C.
Fig. 2. Detail of proximal region of membrane from the same (18° C.).
Fig. 3. Wing lobe of 5th-stage larva produced at 25° C.
Fig. 4. Detail of membrane in the same region as fig. 2 (25° C.).
Fig. 5. Wing lobe of 5th-stage larva produced at 34° C.
Fig. 6. Detail of membrane in the same region as fig. 2 (34° C.).

THE TOXICITY OF POTASSIUM CYANIDE TO TROUT

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(With Five Text-figures)

INTRODUCTION

Lack of fundamental information on the factors determining the survival of fish in water containing poisonous substances has led to the use of a variety of experimental techniques and methods of interpreting results in studies on the toxicity to fish of constituents of waste waters discharged to streams; information on the survival times of fish in solutions where they survive for longer than one or two days is very scanty, and a programme of laboratory studies on the survival of rainbow trout in solutions of cyanide has therefore been started with the object of providing background knowledge for studies of this kind.

In nearly all the toxicity tests reported in the literature, fish have been immersed in a fixed volume of poisoned water, but both Allee & Bowen (1932), using a suspension of colloidal silver, and Carpenter (1927), using solutions of lead nitrate, have shown that when fish are kept in the test solution the concentration of poison falls and survival time can be increased by increasing the number of fish in the solution. From their experiments these workers concluded that the reduction in concentration of poison was partly due to precipitation by mucus. It is probable that similar reactions reduce the concentration of many lethal agents and this, together with other changes produced in the water by the metabolism of the fish, would result in a continually changing environment during a test with a fixed volume of solution, so that the survival times could not be assumed to be representative of those of fish in a continuously polluted river. The simplest way to overcome this difficulty is to test the fish in a vessel through which a stream of poisoned water flows at such a rate that the activity of the fish cannot seriously alter its composition. Such an apparatus has been used by Erichsen Jones (1938), but it was too small for more than two minnows to be tested at one time. It is desirable to test more fish than this in one concentration if variations in resistance of fish to a poison are to be assessed. It was therefore decided to make tests in a vessel in which fifty yearling rainbow trout could be tested together in a stream of water in which concentration of poison, dissolved oxygen tension, and temperature were kept as constant as possible.

MATERIALS AND METHODS

Apparatus

A constant concentration of poison is maintained by adding a solution of poison at a constant rate (approximately 2 ml. a minute), to a stream of dechlorinated and oxygenated tap water flowing at a constant rate of about 2 l. a minute, mixing the

two streams thoroughly, and passing the poisoned stream through a test tank holding 200 l., in which the water is thermostatically controlled to the required temperature. Fig. 1 is a flow diagram of the apparatus. Mains water is passed through a sand filter to remove suspended solids and then through a tower filled with activated carbon to remove any chlorine. The treated water is oxygenated by pumping at high velocity through a glass filter pump into a storage tank. From

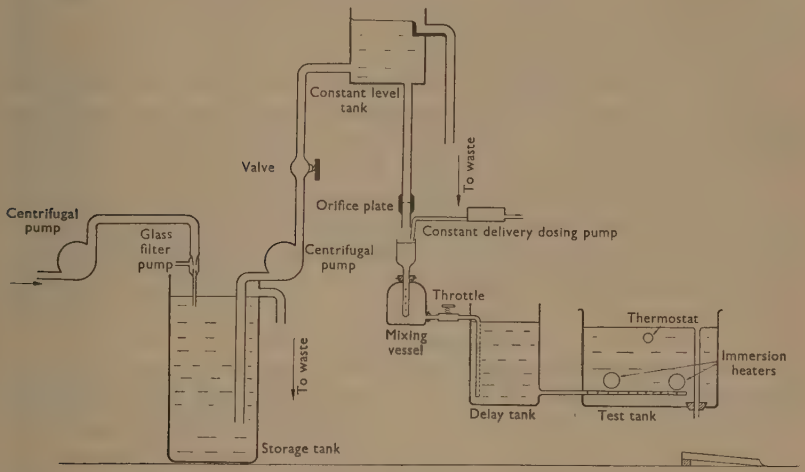

Fig. 1. Flow diagram of the apparatus used to test fish in a stream of water containing a constant concentration of poison at a controlled temperature. (Not to scale.)

the storage tank the water is pumped up to a constant level tank which provides a constant head above an orifice plate in the outlet pipe and controls the flow of water to a constant rate close to 2 l. a minute.

Water from the constant level tank falls into the receiver of the mixing vessel into which a solution of poison is added at a known rate by a constant delivery dosing pump. Thorough mixing is effected by passing the water and the solution of poison through small holes in the central pipe of the mixing vessel. From the mixing vessel the water flows through a throttle to a delay tank of 50 l. capacity, in which any small fluctuations in concentration of poison are smoothed out, and from there it flows into the heavily glazed iron test tank. The inlet pipe forks into two branches which run along the base of the tank close to each of the long walls, and the poisoned water enters the tank through small holes in the walls of the pipes. The water in the tank is heated by two 2 kW. immersion heaters, heavily plated with tin, and the temperature is controlled by a thermostat of the bimetal spiral type enclosed in a tinned metal pocket projecting into the water through the side of the tank.

PERFORMANCE OF THE APPARATUS

Maintenance of constant concentration of poison

The rate of flow of water through the mixing vessel will remain within ±0·33% of the mean rate for many weeks, and the variations in delivery rate of the dosing pump rarely exceed ±1·4% of the mean rate. In one test a solution of Rhodamine B, a fast dye which can be determined photoelectrically in concentrations of about 1·0 p.p.m. to within ±2·32%, was added to give 1·0 p.p.m. in the test tank; the results of nine determinations made over a period of 21 hr. lay within ±2·78% of a mean value of 0·99 p.p.m.

Maintenance of the required concentration of cyanide in the test tank is often complicated by an apparent loss of cyanide when this is determined by analysis. The amount lost varies with different samples of water, and the cause of this cyanide demand is not known; it is usually observed with the tap water used in this laboratory and with distilled water. All cyanide determinations reported in this paper were made by a modification of Robbie's (1944) phenolphthalein method (Merkens, in preparation). With this modification, concentrations of cyanide between 0·2 and 0·03 part cyanide per million can be determined within approximately ±4%.

Dissolved oxygen concentration

Measurements were made over a 3-day period and at a temperature of 15° C., of the dissolved oxygen tension in water entering and leaving the test tank; fifty small rainbow trout were in the tank throughout. The dissolved oxygen in the inflowing water varied from 100 to 105% of saturation (mean 102·7%) and in the outlet from the test tank it varied from 93 to 101% of saturation (mean 95·9%).

Temperature control

The temperature in the test tank can be controlled to within ±0·5° C. Except where otherwise stated, all experiments reported in this paper were made at 17·5±0·5° C.

pH *value*

No pH control is incorporated in the apparatus as at present used; the pH value of the water varies between 7·4 and 8·0. Wuhrmann & Woker (1948) have shown that the toxicity of cyanide to fish is affected by the proportion of the cyanide ion to the undissociated molecule, HCN being more toxic than the equivalent concentration of hydrogen and cyanide ions. According to Wuhrmann & Woker about 97 to 93% of the hydrogen cyanide produced by hydrolysis of the added KCN will be in the undissociated condition in a pure solution between pH values of 7·4 and 8·0. The presence of other ions in the solution will modify these proportions, but random fluctuations of pH value within these limits should not greatly alter the toxicity of cyanide.

Test animals

All the experiments reported were made with rainbow trout between 9 months and 2 years old, obtained from a commercial trout farm. The fish were kept in the laboratory in aquaria supplied with running, continuously aerated, dechlorinated

tap water; the temperature of the water was not controlled. The fish were fed once daily on finely minced bullock's heart.

Before every test in cyanide solution the fish to be used were removed from their stock aquarium to another which was maintained at the temperature of the experiment (17·5° C.). Except in Exp. 4, they were not fed from the time they entered the acclimatization tank until the end of the experiment.

In all tests which were completed in 9 hr. or less (that is in concentrations of 0·14 part cyanide per million or more) the fish were watched continuously; in tests of longer duration they were observed only at intervals, but the interval was never greater than 1 hr. except for the last surviving fish in the test in 0·09 part CN per million in Exp. 3. In those experiments where the fish were continually observed, the time from immersion in the poisoned water until each fish had overturned and remained motionless on its back or side for 5 sec. was recorded. Experience has shown that after a fish has remained overturned for 5 sec. it rarely regains equilibrium while remaining in the poison, unless disturbed. Fish which had overturned were taken from the test tank and placed in well-oxygenated unpoisoned water. Fish removed immediately usually regained equilibrium in a few minutes and showed no ill effects from the poison, but in the experiments where the fish were observed at intervals they were often dead when first seen to be affected, and those which were overturned but still alive did not always recover when transferred to unpoisoned water. In these cases the survival time was taken as the time in minutes from the beginning of the experiment until the time when the fish was first seen to be overturned or dead.

EXPERIMENTAL

Selection of acclimatization period

To reduce errors caused by variations in environment, conditions, such as temperature, in the test tank were standardized as far as possible. To keep the temperature in the test tank constant throughout a long series of tests, the experimental temperature has to be above that of the mains water supplying the aquaria, so if fish were transferred direct from aquaria to test tank they would be subjected to abrupt temperature changes. Sumner & Wells (1935) have shown that the resistance of fish to cyanide and some other poisons at one temperature is affected by the difference between the temperature of the aquaria in which they have been living and the test solution. This source of variation could be avoided by keeping all aquaria at a constant temperature, but since facilities for this are not available the fish were acclimatized for a period at the experimental temperature before each test. In Exp. 1 the effect was studied of different periods of acclimatization on the overturning time of rainbow trout in cyanide, so that a suitable period for acclimatization could be chosen.

Eighty young rainbow trout (mean length 8·98 cm.; standard deviation 0·94 cm.) were graded for size, and sorted into two batches as nearly as possible equivalent with respect to length. The tank used for acclimatization was divided by a partition perforated with holes through which fish could not pass. A batch of fish was kept

in each half of the tank, so both were subject to similar environmental conditions. Batch A was immersed in 0·15 part CN per million after spending 24 hr. in the acclimatization tank. When each fish overturned the period of survival before overturning was recorded and it was replaced in the acclimatization tank. This batch remained in the acclimatization tank until the last day of the experiment when it was tested again. Batch B was tested in the same way after acclimatization for 48 hr. and again on three subsequent days. The fish were returned to the acclimatization tank after each test. During the experiment five fish were lost from batch A and two from batch B.

Results of this experiment are summarized in Table 1.

Table 1. *Effect on resistance of rainbow trout to potassium cyanide of increasing time of acclimatization to the temperature of the tests* (17·5° C.)

Batch of fish tested	Period of retention in acclimatization tank before immersion in cyanide solution (hr.)	No. of fish available for test	Cyanide concentration required (parts CN per million)	Mean of cyanide concentrations obtained by analysis (parts CN per million)	Mean period of survival (min.)
A	24	36	0·150	0·150	28·81
B	48	40	0·150	0·151	28·60
B	97	40	0·150	0·149	35·15
B	120	40	0·150	0·149	46·97
B	172	38	0·150	0·150	39·68
A	191	35	0·150	0·150	50·80

Survival time increases with the duration of the acclimatization period. A regression analysis (Emmens, 1948) shows that the increase is highly significant statistically, while the deviations from the best fitting straight line are not significant at the 0·02 probability level. There is no evidence of a difference between batches A and B, so it is rather unlikely that the increasing resistance of the fish to the cyanide was due to repeated exposure to it. It is possible that if the trout had been acclimatized for longer periods their resistance to cyanide would have reached a steady state, but evidently more than 8 days are required for this. Practical considerations necessitated an acclimatization time of not more than a few days, and since it appears from Exp. 1 that differences in the duration of acclimatization would produce varying resistance to cyanide, a 2-day period of acclimatization was adopted as standard practice, and in the experiments that follow all tests were started as nearly as possible 48 hr. after the fish were put into the acclimatization aquarium.

Length of fish and resistance to cyanide

Yearling rainbow trout vary considerably in size even when they have all been reared under similar conditions. Exp. 2 was made to determine whether resistance to cyanide was correlated with body length.

All the fish used were taken from one pond at the trout farm. The supplier said they were all of the same age and had been reared under the same conditions since

they were spawned. Sixty-three 1-year-old rainbow trout ranging in length from 5·5 to 17·25 cm. were kept without food in running water at 17·5° C. for 48 hr. before the experiment, and were then immersed together in the test tank through which flowed a stream of water to which potassium cyanide was added to bring the concentration to the equivalent of 0·16 part CN per million. The cyanide demand of the water reduced this to an average value of 0·153 part CN per million (maximum 0·154 p.p.m., minimum 0·152 p.p.m.). The periods of survival of fish of different lengths are shown in Table 2. There is an obvious tendency for the longer fish to succumb sooner than the shorter fish.

Table 2. *Relation between length of yearling rainbow trout and period of survival in potassium cyanide*

(Length of each fish measured to nearest 0·25 cm.)

Range of length (cm.)	No. in group	Mean length (cm.)	Mean period of survival (min.)
5·5– 6·25	3	5·75	39·0
6·5– 7·25	11	7·13	37·0
7·5– 8·25	7	7·93	33·4
8·5– 9·25	10	8·96	24·1
9·5–10·25	8	9·81	22·25
10·5–11·25	3	11·00	28·3
13·5–14·25	1	14·00	12·0
14·5–15·25	13	15·00	18·4
15·5–16·25	6	15·71	16·7
16·5–17·25	1	17·25	16·0

This relationship between the length of a rainbow trout and its survival time in solutions of cyanide is important in the design of toxicity tests, because if the fish to be used in an experiment are selected so that the range of size covered is small, the variance of overturning time will be reduced and the error of statistics such as mean period of survival and median lethal time will be smaller. At the same time, however, the statistics will be less generally representative of the fish of the age group tested. It would be worth while using fish of as nearly as possible the same size to increase the sensitivity of an experiment where the effect of dissimilar treatment was to be compared, but not where the results will be required to show the range of variation typical of a more natural population.

By doing separate tests on various length groups, or by recording the length with the survival time of each fish, results typical of a wider range of length and giving accurate comparison between groups could be obtained.

Distribution of resistance to cyanide and the relation between cyanide concentration and survival time

Exp. 3 was designed to investigate the relation between concentration and survival time over the range 2·0 parts to 0·07 part CN per million and to provide information from which the distribution of resistance to the cyanide concentrations used could be deduced.

Material and experimental design

It was not possible to obtain a large enough stock of fish representative of the range of length typical of one age group without drawing them from different sources and entailing the risk that differences due to environment would be confounded with those due to length. We therefore decided to obtain all fish from one source and to restrict the range of length as much as possible.

Rainbow trout, about 9 months old and approximately equal in size, were selected from one pond at the trout farm where they had been reared together; their mean length was 9·52 cm. (standard deviation 0·98 cm.). The fish were grouped into five classes according to length, and each class was sorted at random into twenty aquaria where the fish were kept until used in the experiment. In this way the stock was divided into twenty samples nearly equivalent with respect to length and with all properties other than those associated with length distributed at random. The fish were grouped in this way to make the batches more alike in resistance to cyanide, but it causes the significance of the differences between batches to be underestimated to a slight extent.

All batches were acclimatized to the experimental temperature (17·5 ± 0·5° C.) for 48 hr. before the start of each test and were not fed until the end of the test. The experimental design provided for twelve tests in concentrations between 2·0 parts and 0·07 part CN per million and one control in unpoisoned water. The other seven batches were held in reserve to be used if a test batch had to be discarded for any reason. The batches were used and the thirteen concentrations were tested in random order. For most tests the fish were immersed together in the test tank and the time of survival of each fish was recorded, but in the test in 1·0 part CN per million the fish overturned so rapidly that not all the survival times could be recorded. When the tests were performed in 2·0 parts and 0·3 part CN per million, the fish were immersed five at a time.

The concentrations of cyanide used, and the values obtained by analysis during each test are summarized in Table 3.

About halfway through the series of tests one batch of fish was immersed in a stream of unpoisoned water at 17·5 ± 0·5° C.; they were not fed. The first fish died after 30 days and 50% had died after 68 days. Since no test in cyanide lasted more than a fortnight, no compensations for natural mortality were made.

Distribution of resistance to cyanide and the relation between the means and variances of survival times

When the percentage of fish overturned in any one of the concentrations of cyanide tested is plotted against time, the points lie approximately on a sigmoid curve. Bliss (1937) has shown that such sigmoid time-mortality curves can be described by assuming that the individual survival times or functions of these times such as the logarithms or reciprocals are normally distributed. The examination of the data of this experiment by the usual statistical techniques and tests would be strictly valid only if the survival times, or some functions of the survival times,

satisfied two criteria; namely, the distribution of the times or transformed times should be normal within each concentration, and the variances of the distributions in the different concentrations should be equal after allowing for the variation due to random sampling.

Table 3. *Concentrations of cyanide added in Exp.* 3 *and determined by analysis in the test tank*

Concentration added (parts CN per million)	Mean of concentrations obtained by analysis (parts CN per million)	Cyanide lost (p.p.m.)	No. of determinations made during test	Coefficient of variation of concentrations obtained by analysis (%)
2·0	1·966	0·034	3	2·39
1·0	0·965	0·035	2	0·73
0·3	0·293	0·007	3	1·67
0·25	0·226	0·024	2	2·52
0·20	0·197	0·003	3	0·78
0·18	0·160	0·020	8	0·53
0·16	0·160	0·000	5	1·13
0·14	0·132	0·008	7	1·49
0·10	0·0846	0·0154	74	3·58
0·09	0·0736	0·0164	61	9·18*
0·08	0·0719	0·0081	70	1·95
0·07	0·0598	0·0102	132	2·98

* Large coefficient of variation probably caused by unusually large fluctuations in cyanide demand of the water.

There are two ways of examining the first criterion. In the first place, probit curves can be drawn. In Fig. 2*a* the survival times recorded during the test in 0·14 part CN per million are plotted as a sigmoid time-mortality curve, and in Fig. 2*b* the probits corresponding to the percentage of fish overturned are plotted against time, log time, and 1/time. If overturning times were normally distributed, the points in the probit-time graph would lie on a straight line, but since the plots of both times and 1/times against probits are curved, and the logarithms of times alone are reasonably linear, it can be concluded that log-survival times are best fitted by the normal curve. The second method is to examine the goodness of fit mathematically; this has been done for each concentration, except 1·0 part CN per million where insufficient individual times were recorded, and the results are summarized in Table 4. The tests employed were the $\sqrt{\beta_1}$ and α tests for skewness and kurtosis (Geary & Pearson, 1938). At the 5% level of significance the survival times and their reciprocals were consistent with the assumption of normality in only one and three concentrations respectively. The logarithmic transform departs significantly from normality in only five of the eleven concentrations.

As the concentration of cyanide decreases and the survival times of the fish increase, the range of individual survival times also increases. This is shown in Table 5, columns 2 and 3, where the variance of survival times increases with mean survival time. The reciprocals of times show a similar trend, but it is not apparent with the logarithms of time for concentrations less than 0·25 part per million. The

last nine variances in column 5, Table 5, have been shown by Bartlett's test to be significantly non-homogeneous, that is, they cannot be assumed to be estimates of

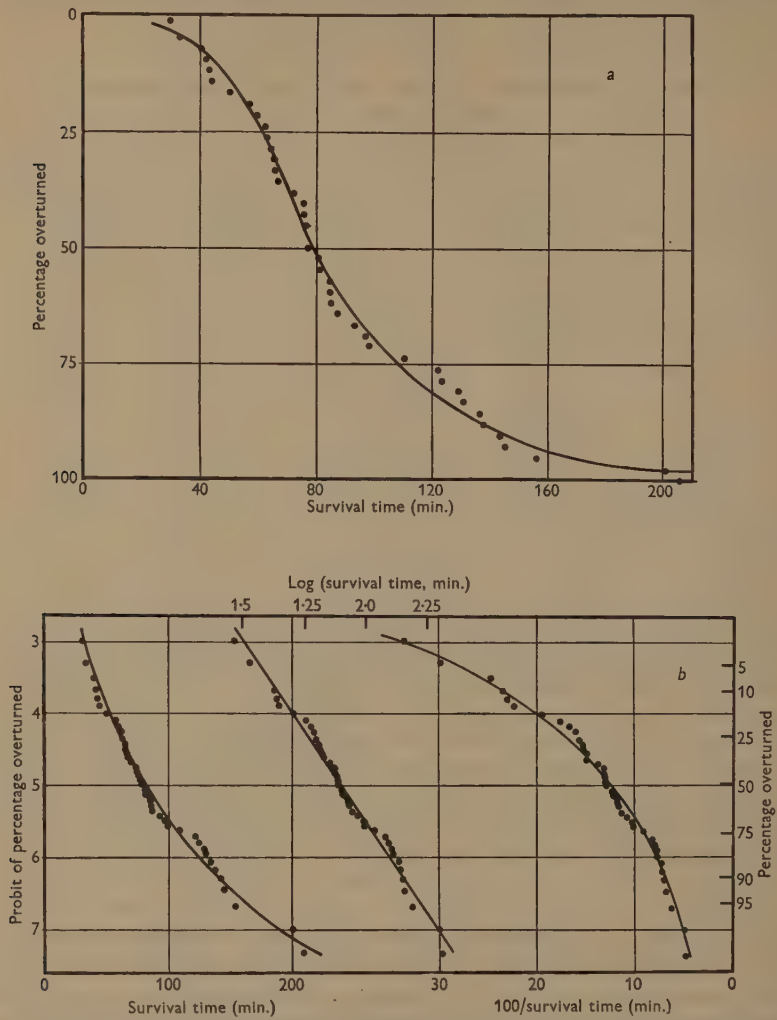

Fig. 2. Survival times of rainbow trout in a solution containing 0·14 part CN per million at 17·5° C. (*a*) Percentage survivors plotted against time. (*b*) Probits corresponding to percentage survivors plotted against time, log time, and rate.

a single population variance of log times common to all the tests. Since they appear to vary at random, however, it is probable that this heterogeneity was caused by variations in conditions not under experimental control.

Table 4. *The fit of normal distribution curves to overturning times, log overturning times, and reciprocals of overturning times*

Concentration (parts CN per million)	N	Time		Log time		1/time	
		$\sqrt{b_1}$	a	$\sqrt{b_1}$	a	$\sqrt{b_1}$	a
2·0	46	−1·2*	0·78	−2·5*	0·69*	4·2*	0·18*
0·3	46	0·6*	0·83	0·1	0·82	0·1	0·81
0·25	31	1·6*	0·69*	1·1*	0·78	−0·3	0·78
0·20	36	1·8*	0·72*	0·9*	0·78	0·2	0·78
0·18	48	1·0*	0·77	−0·5	0·75	1·8*	0·69*
0·16	47	0·2	0·77	−0·1	0·76	2·5*	0·63*
0·14	42	1·0*	0·79	0·0	0·79	1·2*	0·74*
0·10	47	0·6*	0·73*	−1·8*	0·67*	4·4*	0·49*
0·09	40	3·6*	0·58*	0·5	0·77	1·4*	0·74*
0·08	45	0·3	0·76*	−1·2*	0·74*	2·9*	0·63*
0·07	41	1·1*	0·43*	−0·3	0·78	1·9*	0·71*
No. of cases passing both tests		1		6		3	

N = number of individuals in test. $\sqrt{b_1}$ and a = estimates of $\sqrt{\beta_1}$ and α (Geary & Pearson, 1938). In a normal distribution $\sqrt{\beta_1} = 0$, and $\alpha = 0{\cdot}79788$.

* Departures from normality significant at 5 % probability level.

Table 5. *Means and variances of survival times, logarithms and reciprocals of the survival times of rainbow trout in various concentrations of cyanide*

Cyanide concentration (p.p.m.) (1)	Mean of survival times (min.) (2)	Variance of survival times (3)	Mean of log survival times (4)	Variance of log survival times (5)	Mean of reciprocals of survival times (6)	Variance of reciprocals of survival times (7)
2·0	2·66	0·018	0·4178	0·0069	0·3906	$10^{-1} \times 0{\cdot}1040$
0·3	8·84	3·30	0·9376	0·0076	0·1178	$10^{-2} \times 0{\cdot}5616$
0·25	12·41	30·46	1·0627	0·0239	$10^{-1} \times 0{\cdot}9151$	$10^{-3} \times 0{\cdot}7729$
0·20	12·12	37·79	1·0422	0·0319	$10^{-1} \times 0{\cdot}9780$	$10^{-3} \times 0{\cdot}1240$
0·18	24·86	1,655	1·5504	0·0616	$10^{-1} \times 0{\cdot}3389$	$10^{-3} \times 0{\cdot}5666$
0·16	72·40	3,059	1·7482	0·1004	$10^{-1} \times 0{\cdot}2352$	$10^{-3} \times 0{\cdot}4369$
0·14	90·20	1,749	1·9115	0·0384	$10^{-1} \times 0{\cdot}1391$	$10^{-4} \times 0{\cdot}3701$
0·10	2,523·13	1,482,900	3·3308	0·0863	$10^{-3} \times 0{\cdot}6679$	$10^{-6} \times 0{\cdot}9764$
0·09	1,617·50	1,174,610	3·1507	0·0448	$10^{-3} \times 0{\cdot}7902$	$10^{-6} \times 0{\cdot}1498$
0·08	3,600·51	1,649,100	3·5242	0·0322	$10^{-3} \times 0{\cdot}3310$	$10^{-7} \times 0{\cdot}3604$
0·07	4,441	3,996,400	3·5675	0·0337	$10^{-3} \times 0{\cdot}2555$	$10^{-7} \times 0{\cdot}1644$

The joint evidence of the two criteria is that the log transform is markedly superior to the other two forms, but since even the log times depart from the symmetrical homogeneous form more than would be expected from random sampling, the hypothesis of log normality with uniform variance cannot be unquestionably accepted. It is known, however, that the departures from the two ideal conditions already stated have to be considerable before conclusions drawn from the statistical analysis are invalidated. It is extremely unlikely that the departure of the log transform from the normal homogeneous form is sufficiently serious to vitiate the inferences to be drawn from the analysis, as all are shown to exist at a very high level of statistical significance.

Relation between concentration of poison and survival time

Where the logarithms of the survival times of fish immersed in a solution of poison are normally distributed the best estimate of the median survival time, or time taken to overturn 50% of the test fish, is given by the geometric mean of the survival times

$$\text{Median survival time} = \text{antilog}\,\frac{(\Sigma \log T)}{N}, \qquad (1)$$

where T is the survival time of each fish, and N is the number of fish in the test.

In Fig. 3 the median survival times estimated from each test by equation (1) are plotted against the concentrations of cyanide added to the test tank, in both cases on logarithmic scales. If the mean concentrations of cyanide obtained by analysis are plotted instead of the concentrations of cyanide added a very similar curve is obtained but the position and slope are slightly different. Over most of the curve, logarithms of concentration and logarithms of survival time are linearly related by the line A–A^1, but at the higher concentrations the points diverge systematically from it.

The equation for the line A–A^1 is

$$n \log C + \log T = \log k, \qquad (2)$$

or

$$C^n T = k, \qquad (2a)$$

where C is concentration of cyanide in parts per million; T is survival time in minutes; and n and k are constants.

A regression analysis has been carried out for the data corresponding to concentrations less than 0·2 p.p.m.; the constants of the best fitting straight line are $n = 5{\cdot}639$ and $\log k = -2{\cdot}700$. Both the slope of the line and the departures of the group means from the line are highly significant statistically ($P < 0{\cdot}1\%$ in each case). Since the group means are scattered about the line at random over this region, with no systematic trend away from it, equation (2) can be assumed to represent the relation between C and T, and the results of the regression analysis to show that errors in the estimation of T are mainly due to factors acting at random which increase or decrease the survival times of all the fish in a batch, while the errors due to the variation of survival times within a batch are relatively insignificant. Inaccuracies in the maintenance of the required concentration of cyanide could account for some of this error, and factors associated with differences in environment between the stock aquaria, the different dates on which the tests were started, or uncontrolled variations of the chemical and physical properties of the water affecting the response of the fish might also contribute to it.

Because deviations of group means from regression constitute the major source of error in the experiment, the significance of such departure from the line as is shown by the highest concentrations must be assessed from the scatter of median survival times and not from the deviations of individual log survival times from the group means. The dotted lines at either side of A–A^1 (Fig. 3) represent the limits within which 95% of the median survival times should lie over the range of con-

centrations for which equation (2) holds. They have been calculated on the assumption that the constants of the line are known without error. To avoid including points near the region where A–A^1 meets B–B^1 the five shortest median survival times were not used when calculating these limits. All points except those for concentrations

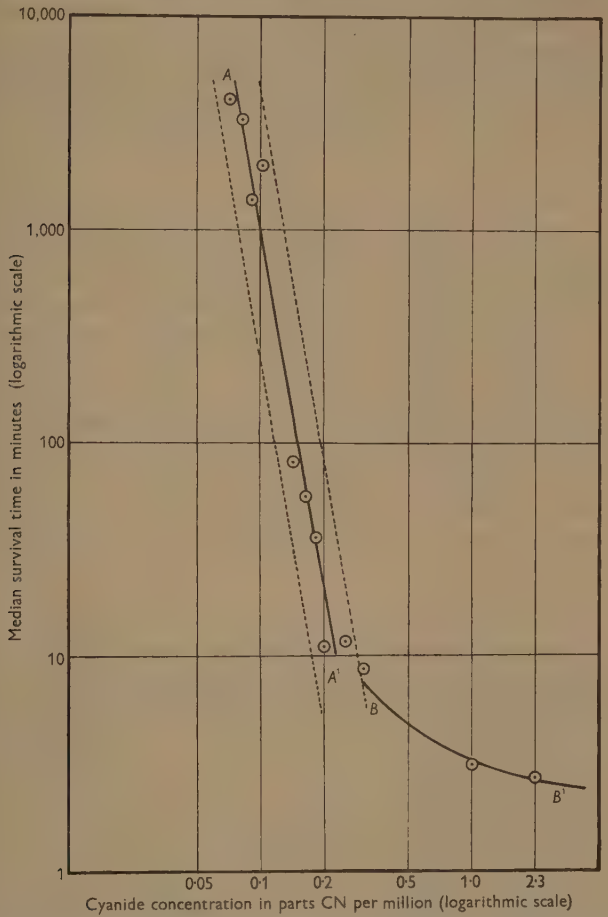

Fig. 3. Relation between concentration of cyanide and survival time of rainbow trout at 17·5° C. The points represent the median survival times and the dotted lines 95 % confidence limits calculated from the scatter of the seven longest median survival times about the fitted line. A–A^1 and B–B^1, for explanation see text.

of 0·3 part CN per million and more lie within the band; there is therefore no significant departure from equation (2) between 0·25 and 0·07 part CN per million, and the intersection of A–A^1 and B–B^1 presumably occurs between 0·25 and 0·3 part CN per million.

Factors associated with the varying resistances of trout to cyanide

The differing resistances of individual rainbow trout to cyanide at the time of any one test has been shown in Exp. 2 to be due in part to a relation between lengths and resistance but some could be due to other inherent and permanent differences between individuals, to short-lived random effects of the environment, or to a combination of both these factors. In Exp. 4, which was designed to estimate the relative importance of these possibilities, use was made of the rapid recovery of trout from cyanide poisoning if they are returned to unpoisoned water shortly after overturning.

Method

Fifty rainbow trout were distinguished by cutting different combinations of notches in the borders of the fins; after marking they were kept in an acclimatization aquarium at 17·5° C. until the end of the experiment. They were not fed during a period of 48 hr. before each test. Eight tests were made over a 3-week period. In each test the fish were immersed in a potassium cyanide solution containing 0·15 part CN per million and the overturning time and markings of each fish were recorded. Losses of fish from a variety of causes during tests 1–7 reduced the number of fish to thirty-eight and another loss reduced the number available in test 8 to twenty-six. When the experiment was completed it was found that there was no significant correlation between the overturning times of the trout and the number of notches cut, so presumably this method of marking does not affect the resistance of fish to cyanide.

Results

There was an obvious tendency for any one fish to retain its original position in the overturning order throughout the series of tests, although the order was never exactly the same on any two occasions. The results of the experiment are summarized in Table 6. The correspondence between the survival times of individuals during the series has been assessed by calculating the product moment correlation coefficients (r) between the overturning times in test 1 and the times in tests 2–8; in every case the correlation is very significant.

Since there was not an absolute correspondence between the overturning times in the eight tests, the resistance of each individual cannot be due entirely to permanently inherent factors, but the stable and significant correlation between overturning times shows that such factors, persisting for at least 3 weeks, have a role in determining the survival time of an individual; the actual overturning time is the resultant of permanent, intrinsic factors and short-lived effects of the environment. It can be shown by the reasoning in Peters & Van Voorhis (1940, p. 120 *et seq.*) that the correlation coefficients in this experiment have the meaning

$$r = \frac{\sigma_i^2}{\sigma_i^2 + \sigma_e^2},$$

where σ_i^2 is the variance due to the intrinsic causes persisting in the fish through the series of tests, and σ_e^2 is the variance due to the other factors which have modified the inherent resistance. The proportion of the variance of survival time due to the intrinsic factors in this experiment is between 66·14 and 84·28%.

Table 6. *Summary of results of experiment to determine the extent to which the varying resistances of rainbow trout to potassium cyanide are due to inherent and permanent differences between individual fish*

Test no.	Time from start of 1st test (hr.)	Cyanide concentration		Mean period of survival (min.)	Correlation coefficients between the overturning times		N
		Desired (parts CN per million)	Mean value by analysis (parts CN per million)		Between tests	r	
1	—	0·150	0·152	19·93	—	—	—
2	3½	0·150	0·155	18·64	1 and 2	0·84	38
3	24	0·150	0·149	24·56	1 and 3	0·84	38
4	48½	0·150	0·150	29·90	1 and 4	0·74	38
5	51	0·150	0·151	25·72	1 and 5	0·74	38
6	72	0·150	0·150	28·30	1 and 6	0·78	38
7	192	0·150	0·140	43·03	1 and 7	0·66	38
8	507¼	0·150	0·157	51·76	1 and 8	0·71	26

r = product moment correlation coefficient; N = number of pairs of observations used in calculating r.

DISCUSSION

The method usually employed to interpret the results of toxicity studies with fish derives from that used by Powers (1917), who concluded from a study of many toxic substances to goldfish that the shape of the concentration-survival time curve resembles a rectangular hyperbola, and that with most poisons there is a range of concentrations where the relation between concentration and 100/survival time is linear, but if the data are sufficiently extensive the points usually depart from the straight line at the higher and lower concentrations. Our data from Exp. 3 are plotted in this way in Fig. 4, except that 100/median survival time has been used in place of 100/time taken to overturn each fish. The points lie on a sigmoid curve similar to that found by Powers with most of the poisons he investigated, and, in accordance with his practice, the dotted straight line is fitted to the central part of the curve where the slope is steepest. Powers was exploring the possibility of using the survival times of fish in toxic solutions to assay an unknown concentration of poison by comparing its toxicity with those of known concentrations, and did not investigate the departures from the straight line in any detail, but this method has often been used by other authors to interpret the results of toxicity tests designed to assess the danger to fish of a poison to be discharged into a river. Often the only concentrations tested are within the range for which the linear relation holds, and if the straight line is extrapolated it cuts the concentration axis. Such extrapolation can be very misleading; if there had been no data for our five lowest concentrations it would appear that rainbow trout should not die in concentrations of less than

0·14 part cyanide per million, which is not the case. The method also discards any information the experiment may give about the effect of concentrations below the point of intersection, although information on the more protracted survival times in low concentrations is important in toxicity studies with fish, as a continuous pollution which will kill fish in several weeks could be as harmful to a fishery as one which would kill them in a few minutes or hours.

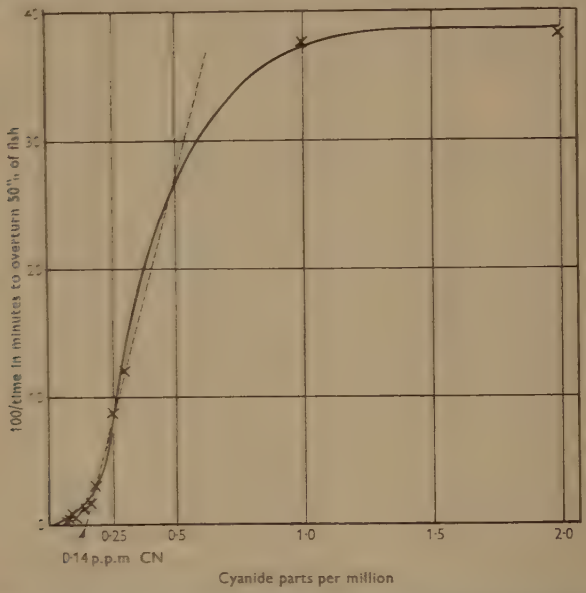

Fig. 4. Relation between concentration of cyanide and reaction time of fish plotted according to the method of Powers (1917).

Equation (2), however, describes the relation between cyanide concentration and median survival time both for the lower concentrations for which concentration appears to be linearly related to 100/survival time, and for the still lower concentrations we have tested; it does not imply the existence of a threshold toxic concentration.

When the logarithms of the survival times of fish in one concentration of poison are normally distributed the expected survival time of any percentage P is given by

$$\text{Expected survival time of } P\,\% = \text{antilog}\,\{\overline{\log T} + \sigma(y-5)\},^{*} \qquad (3$$

where $\overline{\log T}$ is the mean of the logarithms of the survival time, σ is the standard deviation of the logarithms of the survival times, and y is the probit corresponding to percentage P from Emmens (1948, Table 14.3).

The variances from which the standard deviations of the log survival times in

* This equation is derived from that given by Bliss (1937).

ncentrations between 0·25 and 0·07 part CN per million are derived differ gnificantly from one another but show no systematic increase or decrease with eir means. Had they been homogeneous, a better estimate of standard deviation er this range could have been made from their mean, than by calculation from e survival times in any one concentration; $\bar{\sigma}$ could be substituted for σ, and g $k-n \log C$) for $\overline{\log T}$ in equation (3) to give

$$\text{Expected survival time of } P\ \% \text{ at cyanide concentration } C = \text{antilog}\{\log k - n \log C + \bar{\sigma}(y-5)\}. \quad (4)$$

is calculated from the equation

$$\bar{\sigma} = \sqrt{\frac{\Sigma\Sigma\,(\log T_i - \overline{\log T_i})^2}{\Sigma(N_i - 1)}},$$

ere T_i is the survival time of each fish in the ith test, and N_i is the number of dividuals in the ith test.

Since the variances are heterogeneous, a value for $\bar{\sigma}$ calculated from the data of p. 3 is not the best estimate of a 'true' population standard deviation common all the tests, but an intermediate value of significantly different standard deviaons, no one of which is more likely to be representative of the whole series than y other. Equation (4) has been used to calculate the lines relating concentration d the survival times of 25 and 75% of the test fish; these are shown in Fig. 5 d the times observed during the tests at which these percentages were surviving e plotted for comparison. The general agreement between the data and equaon (4) suggests that if it were possible to ensure identical conditions apart from anide concentration in all tests and identical environmental histories for all the tches of fish, departures from equation (4) might be due only to sampling the h at random. If this is so, better standardization of the environments in which e batches are kept before and during a test, and the performance of tests in all ncentrations at the same time should give results in closer agreement with the pothesis.

In biological assays where the survival times of test animals are used the garithms of survival time and the logarithm of dose seem more often to be nearly related than other functions of time and dose (Emmens, 1948, p. 169). stwald (1907) applied the relation $C^n T = k$ to data obtained by studying the rvival of *Gammarus* in different dilutions of sea water. Chick (1908) found that is relation held for the concentration of several disinfectants and the time taken kill bacteria. Perry (1950) found that the dose of neoarsphenamine and the rvival time of mice followed this law. Ostwald's equation seems applicable to e effect of many drugs and poisons on a great variety of living organisms, and appears to be true for the effect of many poisons on fish. Although Powers used e $100/T$ method to interpret his results, his data for the toxicity of substances to oldfish are well fitted by Ostwald's equation in fourteen of the sixteen cases we ave examined, and there is no systematic departure from the law at the lower ncentrations, although in some the points depart from the line at the higher ncentrations similarly to the points along B–B^1 in Fig. 3.

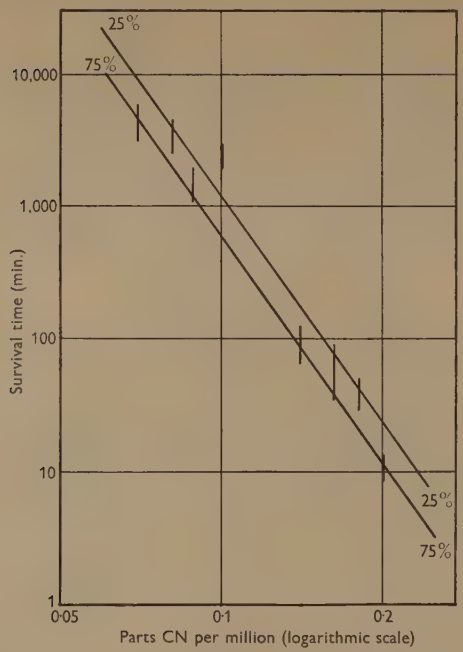

Fig. 5. Relation between concentration of cyanide and time taken to overturn 25 and 75 % of rainbow trout at 17·5° C. plotted on logarithmic scales. Continuous lines computed from equation (4); the short vertical lines join the times at which 25 and 75 % of the fish were observed to overturn during the tests.

SUMMARY

1. An apparatus is described in which fifty yearling rainbow trout can be tested in a stream of water at a constant temperature and containing a constant concentration of poison.

2. Exposure to cyanide causes the fish to lose equilibrium control and to turn over. Resistance is measured as survival time, by which is meant the time taken to overturn the fish.

3. When the experimental temperature is higher than that of the water in the stock aquaria the resistance of yearling rainbow trout to cyanide increases with increasing time of acclimatization to the temperature of the experiment.

4. Small yearling rainbow trout tend to be more resistant to cyanide than larger fish of the same age.

5. The distribution of the logarithms of the survival times of the test fish in cyanide solutions, though not precisely normal, is sufficiently close to a normal distribution to justify the application of standard statistical techniques. Neither the distribution of the survival times nor that of the reciprocals of the times provide a suitable basis.

6. At 17·5° C. in the range 2·5 parts to 0·07 part CN per million it has been found that (i) mean log survival time decreases linearly with log concentration, (ii) the variance of log survival time is approximately constant, and (iii) the distribution of log survival time is approximately normal. At higher concentrations these conclusions are not true.

7. The resistance of an individual rainbow trout to cyanide is mainly determined by inherent properties which persist for at least three weeks.

We wish to record our thanks to the Director of Building Research for assistance from his statistical staff at various stages of the work. We also wish to thank Mr P. S. Hewlett of the Pest Infestation Laboratory for reading the paper in typescript and for many helpful suggestions.

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REFERENCES

Allee, W. C. & Bowen, E. (1932). Studies in animal aggregations. Mass protection against colloidal silver among goldfishes. *J. Exp. Zool.* **61**, 185.

Bliss, C. I. (1937). The calculation of the time-mortality curve. *Ann. Appl. Biol.* **24**, 815.

Carpenter, K. (1927). The lethal action of soluble metallic salts on fishes. *Brit. J. Exp. Biol.* **4**, 378.

Chick, H. (1908). An investigation of the laws of disinfection. *J. Hyg., Camb.*, **8**, 92.

Emmens, C. W. (1948). *Principles of Biological Assay*. London: Chapman and Hall Ltd.

Erichsen Jones, J. R. (1938). The relative toxicity of salts of lead, zinc, and copper to the stickleback (*Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. *J. Exp. Biol.* **15**, 394.

Geary, R. C. & Pearson, E. S. (1938). *Tests of Normality*. Biometrika Office, University College, London.

Ostwald, W. (1907). Über die Beziehungen zwischen Adsorption und Giftigkeit von Salzlösungen für Süsswassertiere (*Gammarus*). *Arch. ges. Physiol.* **120**, 19.

Perry, W. L. M. (1950). Reports on biological standards. VI. The design of toxicity tests. *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 270. London: H.M. Stationery Office.

Peters, C. C. & Van Voorhis, W. R. (1940). *Statistical Procedures and their Mathematical Bases*. New York: McGraw-Hill Book Co.

Powers, E. B. (1917). The goldfish as a test animal in the study of toxicity. *Illinois Biol. Monogr.* **4**, no. 2, 1–73.

Robbie, W. A. (1944). An improved phenolphthalein technique for the micro-determination of cyanide. *Arch. Biochem.* **5**, 49.

Sumner, F. B. & Wells, N. A. (1935). Some relations between respiratory metabolism in fishes and susceptibility to certain anesthetics and lethal agents. *Biol. Bull. Woods Hole*, **69**, 368.

Wuhrmann, K. & Woker, H. (1948). Beiträge zur Toxikologie der Fische. II. Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. *Schweiz. Z. Hydrol.* **11**, 210.

STUDIES IN THE DEVELOPMENT OF THE RAINBOW TROUT (*SALMO IRIDEUS*)

II. THE METABOLISM OF CARBOHYDRATES AND FATS

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(*Received* 20 *May* 1952)

(With Two Text-figures)

I. INTRODUCTION

The first paper of this series (Smith, 1947) reported the measurement of wet and dry weights, nitrogenous excretion and heat production of a single batch of fertilized eggs of the rainbow trout (*Salmo irideus*). It was shown that the dry-weight loss of egg and alevin was commensurate with the fall in total nitrogen and in total fuel value of the system. The dry-weight losses observed could be attributed to the observed combustion of protein, together with the combustion of some substance with a fuel value characteristic of fat. It was concluded that the main sources from which the energy for development is derived are protein and fat.

In the present paper the results of the determination of total carbohydrate are set out, together with the results of glyceride-fat extraction of dry egg, embyro and yolk material; an analysis of the metabolism of glyceride and phosphatide fats in trout development is presented; the probable substrates used in daily heat production are traced; and the concept of a sequence of energy sources in embryonic development is discussed.

II. MATERIAL

The batch of trout eggs on which carbohydrate determinations were made was also used for the measurement of heat production and nitrogen excretion, and for wet- and dry-weight determinations: details of this batch are given in Smith (1947). About the 22nd day eggs were weighed singly on a torsion balance, and only those with a weight of from 85 to 94 mg. were used for subsequent work. Sampling errors were thereby minimized.

For the determination of the glyceride-fat content dried samples of egg, embryo and yolk of the 1934–5 rearing were used. The egg size, hatching date and period of larval life of these were strictly comparable with those of the 1936–7 batch which is under discussion.

III. METHODS

(*a*) *The determination of total carbohydrate*

With the exception of a single sample of yolk, air-dried before analysis, all determinations of total carbohydrate were made on fresh material. The method used was that of Tsai (1933) of which the essential features are a preliminary hydrolysis with

2·2% hydrochloric acid to release sugars in combination, neutralization with caustic soda, and precipitation by means of mercuric sulphate of any non-carbohydrate substances showing reducing action. Since mercuric sulphate precipitates glycogen, it is essential that the preliminary hydrolysis be complete before adding mercuric sulphate. Excess mercuric ion is removed by barium carbonate and by the action of zinc dust in alkaline solution. Total carbohydrate is then estimated in the filtrate by heating with Murphy and Young's reagent, followed by a titration with iodine-thiosulphate. The reagents used were calibrated with standard glucose solutions and the results of analysis are expressed in milligrams of glucose per gram of wet tissue.

(*b*) *The determination of the glyceride-fat content*

The determinations were on dried samples of egg, embryo and yolk, using a modified Soxhlet extraction apparatus requiring samples of approximately 50 mg., weighed on a microbalance. The extracting agent was carbon tetrachloride, so that the results are comparable with those for the Atlantic salmon obtained by Hayes (1930). The method of extraction using carbon tetrachloride is of limited value, since the agent is a non-polar solvent and will not extract polar lipids such as phosphatides. It was difficult to handle some of the later yolk samples because, when ground in an agate mortar, oily matter was pressed out from the material so that uniformly mixed samples could not be prepared. Extraction was continued until there was no further increase in weight of the extract, collected in weighing bottles and evaporated to constant weight at room temperature, using a 'Hyvac' pump. It proved impracticable to weigh the extraction cones, since the change in weight of these with varying conditions of atmospheric humidity necessitated enclosure in a weighing bottle, and such a bottle would have been too bulky for the sensitive microbalance employed.

IV. EXPERIMENTAL RESULTS

(*a*) *The carbohydrate content of egg, embryo and yolk*

The results of the analysis of total carbohydrate in egg, embryo and alevin are set out in Table 1. Smoothed mean values of the carbohydrate content and the wet weights of egg, embryo and alevin (see Smith, 1947) permit computation of the total carbohydrate content of the system. The results are set out in Table 2. There is no significant difference between the carbohydrate content of fertilized and unfertilized eggs. In the fertilized egg a peak in carbohydrate content occurs about the 9th day when gastrulation overgrowth is almost complete; this is followed by a steady decline from the 9th to the 18th day. Subsequently, a slow synthesis sets in and is interrupted by hatching around the 36th day.

The amount of carbohydrate in the yolk material is very low and cannot be detected. After the 50th day, however, the yolk-sac wall, because of its considerable blood supply, may contain a significant amount of carbohydrate.

A short period of relatively intense carbohydrate combustion occurs on or about the 66th day, and the carbohydrate so consumed is made good by

synthesis at a later stage. This observation is the more remarkable since yolk reserves are almost exhausted at this period and the alevins were denied food. Under normal conditions the young fish would be eating at this stage.

Table 1. *Measurements of total carbohydrate content expressed as glucose*

Age (days)	mg./g. wet material expressed as glucose results of separate determinations	Glucose (mg./g. mean value)	Sample
3	1·86, 1·83	1·84	Fertilized eggs
3	1·49, 1·93	1·7	Unfertilized eggs
7	2·10, 1·95	2·02	Unfertilized eggs
7	1·75, 1·73	1·74	Fertilized eggs
10	2·40, 2·28	2·34	Fertilized eggs
13	1·86, 2·37, 2·46	2·23	Fertilized eggs
18	1·00, 1·00	1·00	Fertilized eggs
23	0·92, 2·00	1·46	Fertilized eggs
27	1·3, 1·1, 1·5	1·3	Fertilized eggs
32	2·14, 2·32	2·23	Fertilized eggs
36	1·89, 0·6	1·3	Fertilized eggs
36	3·4	3·4	Embryo
36	0·75	0·75	Yolk (dried before analysis)
39	3·07	3·1	Embryo
44	4·1, 3·9	4·0	Embryo
49	3·74, 3·44	3·59	Embryo
49	0·00	0·00	Yolk (dried before analysis)
53	3·6, 4·0	3·8	Embryo
59	3·04, 2·99	3·02	Embryo
69	2·42, 2·76	2·59	Embryo
71	1·48, 1·00, 1·33	1·27	Embryo
78	2·99, 3·62, 2·81	3·14	Embryo

Carbohydrates, never present in large amount, are thus consumed during three relatively short phases of development:

(i) Immediately after gastrulation when the blood system is developed; 9–18 days of incubation at 10° C.

(ii) During the period of hatching; 34–36 days at 10° C.

(iii) At the onset of starvation; 66–68th days at 10° C.

(*b*) *The glyceride-fat content of egg, embryo and yolk*

In Table 3 the results of the carbon tetrachloride extraction of dried egg, embryo and yolk material are set out. Because of difficulties in sampling and extracting, maximum values are more nearly correct, and smoothed maxima were used in the preparation of Table 4, indicating the probable content of glyceride fat in embryo and yolk throughout the yolk-sac phase.

The figures in Table 3 show that there is a significant rise in the percentage content of glyceride-fat in the yolk-sac from about the 50th day onwards. This rise can be attributed to the selective absorption of material other than glyceride-fat from the yolk-sac.

The analyses are not sufficiently accurate to permit any definite statement about the occurrence of glyceride-fat synthesis between fertilization and hatching. The values for the total glyceride-fat content, calculated from dry-weight determinations and smoothed maximal fat extractions (Table 4), indicate that significant glyceride-

Table 2. *Calculation of the total carbohydrate content (expressed as mg. glucose/100) using wet weight of egg, embryo and alevin already published (Smith, 1947)*

Age (days)	Wet wt. per 100 eggs (g.)	Glucose mg./g. wet wt.	Total glucose mg./100
3	8·45	1·84	15·5
6	8·45	1·74	14·7
9	8·45	2·73	23·0
12	8·45	2·47	19·7
15	8·45	1·72	14·5
18	8·45	1·15	9·7
21	8·45	1·30	10·9
24	8·45	1·42	12·0
27	8·45	1·30	10·9
30	8·45	1·76	15·3
33	8·45	1·81	15·8

Age (days)	Glucose wet wt. 100 embryos (g.)	Glucose (mg./g. wet wt.)	Embryo glucose	Yolk (wet wt. per 100)	Glucose (mg./g. yolk)	Yolk glucose	Total glucose (mg./100)
36	1·24	3·05	3·78	6·94	0·75	5·21	9·0
39	1·50	3·50	5·25	6·68	0·55	3·68	8·9
42	1·89	3·90	7·38	6·47	0·42	2·72	10·1
45	2·71	3·95	10·7	6·08	0·21	1·27	12·0
48	3·46	3·60	12·5	5·80	0·05	0·29	12·7
51	4·33	3·65	15·8	5·40	0·00	0·00	15·8
54	5·82	3·75	—	—	—	—	21·8
57	7·62	3·35	—	—	—	—	25·5
60	9·00	2·95	—	—	—	—	26·6
63	9·91	2·67	—	—	—	—	26·4
66	10·53	2·39	—	—	—	—	25·2
69	11·11	1·70	—	—	—	—	18·9
72	11·42	1·32	—	—	—	—	15·1
75	11·50	2·50	—	—	—	—	28·8
78	11·39	3·08	—	—	—	—	35·0
81	11·04	3·30	—	—	—	—	36·4

fat catabolism is confined from the 63rd to 80th days of rearing at 10° C., being at a maximum around the 66th day. This confirms Hayes and Ross's (1936) observations on the Atlantic salmon, which show a similar fat-consumption peak at a comparable phase of the yolk-sac period.

(*c*) *The estimation of total fat catabolism*

It is clear that the extraction method does not give any reliable indication of the full extent of fat consumption, and it seems reasonable, therefore, to use the knowledge of total catabolism (observed as heat production), of protein catabolism

Table 3. *Mean values of carbon tetrachloride extractions*

Age (days)	Extract yolk (%)	Extract embryo (%)
1 (eggs)	9·4, 10·0	—
1 (eggs)	14·2 (after HCl hydrolysis)	—
29	8·0	—
34	13·0	8·87
36	13·4	—
38	6·53	11·9
41	6·95	9·20
44	11·7	10·2
47	11·9	7·95
50	13·0	—
53	10·6	—
56	26·0	—
59	24·1	7·73
62	30·7	11·18
65	21·4	9·97
68	26·2	—
77	32·7	9·80
77	—	10·05
86	—	13·89

Table 4. *Calculation of the total glyceride (CCl_4 extractable) fat content. All figures refer to* 100 *individuals*

Age (days)	Dry yolk (g.)	Yolk (% extract)	Total yolk fat (g.)	Dry embryo (g.)	Embryo (% extract)	Total embryo fat (g.)	Alevin total fat (g.)	Change in fat content (g.)
1	3·121	10·0	—	—	—	—	0·312	—
36	2·773	12·4	0·343	0·153	11·5	0·018	0·360	0·048
39	2·736	14·8	0·405	0·179	10·8	0·019	0·424	0·064
42	2·648	16·0	0·424	0·220	10·4	0·023	0·447	0·023
45	2·525	17·4	0·439	0·326	10·2	0·033	0·472	0·025
48	2·398	18·4	0·442	0·458	10·2	0·047	0·489	0·017
51	2·230	19·6	0·437	0·620	10·2	0·063	0·500	0·011
54	1·840	22·4	0·412	0·797	10·5	0·081	0·493	−0·007
57	1·540	26·8	0·412	1·068	10·8	0·115	0·527	0·034
60	1·286	29·5	0·378	1·243	11·1	0·138	0·516	−0·011
63	0·992	31·1	0·308	1·434	11·1	0·159	0·467	−0·049
66	0·607	31·7	0·192	1·624	10·8	0·175	0·367	−0·100
69	0·388	32·0	0·124	1·760	10·4	0·183	0·307	−0·060
72	0·223	32·3	0·072	1·821	10·1	0·184	0·256	−0·051
75	0·128	32·6	0·042	1·818	10·0	0·182	0·224	−0·032
78	0·062	32·8	0·020	1·768	10·4	0·184	0·204	−0·020
81	0·010	32·8	0·003	1·707	11·5	0·196	0·199	−0·005
84	—	—	—	1·630	12·9	0·210	0·210	0·011

(observed as nitrogen excretion) and of carbohydrate consumption (already summarized in this paper), in order to compute that proportion of the measured heat production which cannot be attributed to protein and carbohydrate catabolism. This procedure seems the more reasonable, since dry-weight losses, nitrogen excretion and heat production fit into a coherent picture if this method of computation is admitted (Smith, 1947). The calculation is set out in Table 5. Column 1

Table 5. *Figures all refer to* 100 *individuals*

Age (days) (1)	cal./hr. from N excretion (2)	cal./hr. from carbohydrate combustion (3)	cal./hr. heat production measured (4)	Residual heat production (5)	cal./hr. from glyceride fat (6)	cal./hr. from non-glyceride fats (7)
12	0·047	0·26	0·76	0·45	—	0·45
15	0·105	0·26	0·98	0·62	—	0·62
18	0·216	0·26	0·80	0·32	—	0·32
21	0·290	—	0·96	0·67	—	0·67
24	0·258	—	1·04	0·78	—	0·78
27	0·263	—	0·86	0·60	—	0·60
30	0·392	—	0·45	0·06	—	0·06
33	0·516	—	0·78	0·26	—	0·26
36	0·606	0·42	1·20	0·17	—	0·17
39	0·724	—	1·70	0·98	—	0·98
42	0·868	—	2·30	1·43	—	1·43
45	1·01	—	3·05	2·04	—	2·04
48	1·18	—	3·95	2·77	—	2·77
51	1·34	—	5·33	3·99	—	3·99
54	1·71	—	6·64	4·93	—	4·93
57	2·29	—	8·6	6·3	—	6·3
60	2·30	—	9·8	7·5	2·8	4·7
63	2·60	0·04	11·45	8·9	11·2	−2·3
66	3·22	0·22	11·85	8·41	7·9	0·5
69	3·40	0·30	5·72	2·02	6·7	−4·7
72	3·16	—	9·6	6·4	5·1	1·3
75	2·98	—	14·1	11·1	3·2	7·9
78	3·79	—	11·4	7·6	1·6	6·0
81	3·71	—	10·0	6·3	0·0	6·3
84	3·45	—	8·86	5·41	—	5·41

gives age, column 2 the heat production calculated from the observed protein breakdown, and column 3 the heat production resulting from limited and infrequent carbohydrate combustion. The observed heat production is set out in column 4. The heat production remaining after the sum of entries in columns 2 and 3 has been subtracted from column 4, is given in column 5, and is the heat production not traceable to the combustion of protein and carbohydrate. It is inferred that these values arise from the catabolism of some form of fat.

Since amino-nitrogen does not pass through the chorionic membrane, the heat-production figures calculated from the nitrogen excretion before hatching may be less than the true value. The accumulated amino-nitrogen is released during hatching (Smith, 1947).

It is reasonably certain that some fat catabolism occurs before the hatching period from the 34th to 36th days, but to what extent, and when, cannot be definitely stated on the evidence here presented. After hatching the nitrogen excretion can be observed directly, together with the heat production. It is probable that the

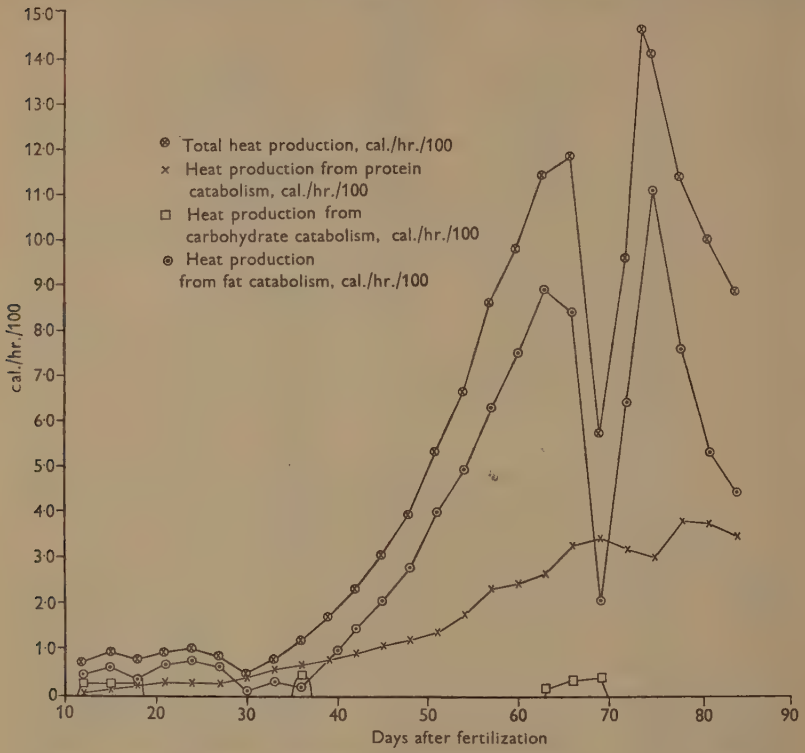

Fig. 1. Graph to show the contribution of carbohydrate, protein and fat consumption to the daily heat production.

figures for the fat catabolism that may be deduced from such directly measured quantities are more reliable than results obtained from any of the methods of total extraction at present available.

The figures from Table 5 for heat production from consumption of carbohydrate (column 3), of protein (column 2) and of total fat (column 5) are set out graphically in Fig. 1 together with the observed heat production (column 4). The total fat consumption is analysed into glyceride and non-glyceride fractions in columns 6 and 7 respectively of Table 5.

It is apparent that, except during the short hatching period, a significant pro-

portion of the daily heat production derives from fat catabolism. Over the major period of development, until at the earliest the 57th day, the fat burnt is non-glyceride, i.e. it is probably phosphatide fat. This non-glyceride fat is probably contained in the aqueous phase of the yolk, bound to the yolk proteins in some form of complex. It may be absorbed and consumed by the embryo in that combination.

Table 6

Age (days)	Heat production in cal./hr. by 1000 cal. of embryo material from the catabolism of			
	Nitrogen compounds	Carbohydrate	Fat	Total
36	0·93	0·64	0·27	1·84
39	0·89	—	1·21	2·10
42	0·83	—	1·38	2·21
45	0·63	—	1·26	1·89
48	0·50	—	1·18	1·68
51	0·41	—	1·21	1·62
54	0·40	—	1·14	1·54
57	0·39	—	1·09	1·48
60	0·34	—	1·10	1·44
63	0·34	0·005	1·13	1·47
66	0·37	0·03	0·96	1·36
69	0·36	0·03	0·21	0·61
72	0·33	—	0·67	1·00
75	0·31	—	1·17	1·48
78	0·41	—	0·83	1·24
81	0·42	—	0·72	1·14
84	0·41	—	0·65	1·07

The relative contribution of the three main substrates, carbohydrate, protein and fat, during the yolk-sac period may be seen more clearly if the intensity of catabolism for each component be expressed in units of embryo tissue. In Table 6 the hourly heat production in calories for each 1000 cal. of embryo is calculated and also the proportionate share of carbohydrate, protein and fat to this hourly heat production. These quantities are represented graphically in Fig. 2. It is clear that the level of catabolism of protein at a high level in the beginning settles down from the 50th day onwards roughly at the level of 0·4 cal./hr./1000 cal. of embryo. This level is maintained throughout relatively violent changes in the level of fat catabolism. Carbohydrate is consumed on two occasions only during the period from hatching to the complete absorption of the yolk-sac, the first over hatching and the other from the 63rd to 69th days of incubation when the thermal contribution would seem hardly significant. It is of interest, however, to correlate this temporary expenditure of carbohydrate reserve with the change-over from combustion of phosphatide fat of the aqueous yolk phase to the glyceride-fat of the yolk oil globule.

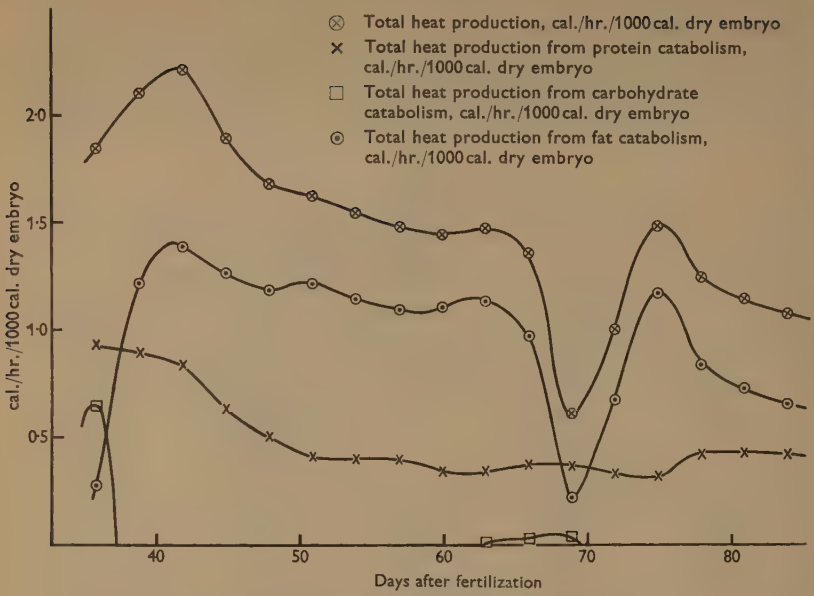

Fig. 2. Graph to show the rate of heat production for each 1000 cal. of embryo arising from the catabolism of carbohydrate, protein and fat.

V. DISCUSSION

The analysis of the fat content of embryo and yolk-sac throughout the development of the rainbow trout egg which has been presented shows that glyceride-fats, which constitute the conspicuous oil drop of the teleost yolk-sac, increase slightly in total amount during the first two-thirds of the free-living yolk-sac phase. After this the glyceride fats are consumed in an intense burst of heat production. The importance of the phosphatide fats for the maintenance of the embryo during the first two-thirds of the yolk-sac phase has been demonstrated. They seem to be coupled to the proteins of the aqueous phase of the yolk. The changes in total carbohydrate show that combustion of this energy source is confined to three periods: (1) immediately after gastrulation; (2) during the period of hatching; (3) at the onset of starvation. The relation of these findings to earlier investigations is discussed below.

(*a*) *The fats of the salmonid egg and embryo*

The various studies of the fat content of salmonid eggs and embryos have shown that unless the fresh yolk is saponified before extraction, the fats obtained are only a fraction of those present. The free fat globules of the yolk, which may be extracted with ether without preliminary hydrolysis, are almost pure glyceride fat; Halpern (1945) indeed reports that the pentane extract of salmon roe (air-dried at 30° C.)

contained pigment but no phosphorus at all. He also experienced difficulty in extracting phospholipids of salmon (*Onchorhynchus*) roe, unless a polar solvent such as methyl or ethyl alcohol was used. 12·5% oil and 6·2% phospholipid were found. It is assumed these percentages refer to the dried material.

Tangl & Farkas (1904) found the ether extract of the trout egg to be 9·4% of the dry weight: in the newly hatched larva the extract amounted to 12·1%. Liebermann's wet saponification at the corresponding stages gave for the egg 21·3% of the dry weight as fat and for the larva 22·8%.

McClendon (1915) studied the egg and 'young' (newly hatched) fish stage of the American brook trout (*Salvelinus fontinalis*) using both the fatty acid determination on fresh material of Kumagawa & Suto (1908) and the alcohol ether extraction of material dried at a low temperature *in vacuo*. His extract was 23·4% of the dry weight of the eggs and 24·7% of that of the newly hatched alevins. An increase of 5·55% in the ether soluble fraction between egg and alevin was also observed, and there was also a parallel increase in fatty acid from 16·17% in the egg to 17·16% in the young fish. The loss in dry weight over the period of development studied was 2·5%, and McClendon considered it probable that fats were formed from protein during this phase of development.

A complete analysis of the fats in the trout egg was made by Fauré-Fremiet & Garrault (1922). Total fats were 22·4% of the dry weight; glyceride amounted to 10%; phosphatides, 8·25%; cholesterol, 1·37%; and unsaponifiable residues, 0·67%.

Hayes (1930) extracted dried samples of *Salmo salar*, eggs and alevins, over a considerable period of their development with carbon tetrachloride. The fat content of the 36-day egg was 12·2% of the dry weight; of the newly hatched alevin at 65 days 8·47% and at 70 days 12·6%. By 116 days, the glyceride-fat content had risen to 20·0% of the dry material of the alevin. The temperature of incubation was low and variable, so these figures may not be compared with those in Table 3, but the rise in glyceride-fat content during the free-living yolk-sac period is very suggestive.

Hayes & Ross (1936) continued this study of Atlantic salmon egg development and found that 22·6% of the egg and 10·8% of the alevin at the end of the yolk-sac period could be extracted by an alcohol ether mixture using fresh material.

Fat analyses of *S. fario* and *S. irideus* material are published by Lafon (1947). He found for a sample of *S. irideus* eggs 15 days before hatching a total lipoid content of 10·04 mg. in an egg of 107·9 mg. wet weight, which is 25·4% of the dry weight. From material of the same batch immediately after hatching the total lipoid amounted to 9·70 mg. in an alevin of 93·01 mg. wet weight, i.e. 28·4% of the dry weight. The proportion of lipoid bound to protein was estimated at about 35% of the total present. Phosphatides were abundant and were roughly 30% of the total lipoid. Free fat was estimated as the ether soluble fraction, the combined lipoid was calculated as the sum of the total extract obtained by ether extraction together with fat extracted by boiling alcohol and anhydrous benzene from the ether extraction residue.

It is of interest that Lafon considers the rate of transfer of the yolk proteins into the embryo more speedy than the transfer of fats. He remarks that the dried material of the yolk-sac becomes enriched in fats as development proceeds. Five days after hatching the total fat content of the yolk-sac is 22·5% of the dry weight, 21 days later it is 34·8% and after a further 9 days no less than 37%. The observed iodine values of yolk and embryo fats showed little variation. The percentage of phosphorus seemed to fall during embryonic development, being slightly less in embryo fats (1·00%) than in yolk fats (1·15%). Lafon suggests that this fall in fat-soluble phosphorus in the embryo might arise either from a preferential oxidation of yolk phosphatides or else from the conversion of phosphatide fat to glyceride fat while in course of transfer from yolk-sac to embryo. The balance of the evidence reviewed here would tend to support this last hypothesis.

The analysis of the rainbow trout egg by Hartmann, Medem, Kuhn & Bielig (1947) is in agreement with the main conclusions reached independently in the course of this study. Their results were as shown in Table 7.

Table 7

	mg. per fresh egg wt. 44·3 mg.	Percentage by weight dry substance	Percentage by weight fresh egg
Dry substance	14·94	100·00	33·82
Water	29·32	0·00	66·18
Ash	0·57	3·81	1·29
Fat (Eiöl)	1·60	10·67	3·61
Phosphatide	1·66	11·09	3·75
Chorion	1·19	7·95	2·69
Protein of egg contents	8·93	59·62	20·16
Total protein (N × 6·25)	—	—	23·19
Nitrogen-free extract	1·03	6·86	2·32

Concerning the phase distribution and chemistry of the major egg components they concluded: 'Das Öl des Eies von *S. irideus* enthält weder Stickstoff noch Phosphor. Die Phosphatide liegen vollständig in der wässrigen Eiflüssigkeit vor. Sie sind an Eiweiss gebunden; ätherlöslicher Phosphor lässt sich erst nach Vorbehandlung mit Alkohol extrahieren. Auch die Kohlenhydrate liegen in gebundener Form vor.'

In the study under discussion the glyceride-fat of the egg was found to be 10·0% of the dry material of the egg which is in substantial agreement with Tangl & Farkas, Fauré-Fremiet & Garrault, and Hartmann *et al.* The value of 12·3% for the glyceride-fat in the newly hatched alevin compares well with the value of 12·1% given by Tangl & Farkas. The fat content at the end of the yolk-sac period (77th day) is 10·05%, in fair agreement with Hayes & Ross's figure of 10·8% for the salmon. Ether extraction of a large number of eggs after hydrolysis in 2·2% hydrochloric acid yielded 14·2% of fatty material showing some release of fat from the yolk as a result of the partial hydrolysis.

To conclude, the evidence reviewed above supports the belief that the fat of the

salmonid egg is distributed between two main sites, one of which is the glyceride-fat globule, and the other a water soluble lipoprotein which constitutes the main bulk of the yolk.

(*b*) *The mechanism of fat absorption*

The yolk-sac wall of the trout embryo is a multinucleate syncytium; it has a well-developed blood circulation in which blood from the liver capillaries after flowing through the yolk-sac wall is collected into a vein leading directly to the heart. Preliminary studies of the egg and alevin have been made, using the lipoid techniques of Baker (1944); they indicate a general distribution of lipoid throughout the bulk of the yolk mass in addition to that of the oil globules. In all stages examined there is evidence that the yolk mass breaks down into a zone of emulsified droplets in contact with the yolk-sac wall, and, at the stages so far studied, such droplets seem to be composed of the bulk lipoprotein phase only. The blood vessels of the yolk-sac contain oily matter staining intensely with Sudan Black. It seems probable, therefore, that after digestion some of the fat at least appears in the embryonic circulation as glyceride-fat.

The glyceride-fat droplets in the yolk-sac may be protected from enzyme action by an adsorbed layer of protein; evidence for this can be seen in the abnormally low interphasial tension (0·6 dyne/cm.) between oil globule and yolk observed by Harvey & Shapiro (1934) and Danielli & Harvey (1934). It is perhaps significant that the lipase activity of aqueous extracts of trout eggs differs notably from the lipase activity of the solids left after water extraction (Falk, Noyes & Lauberblatt, 1927). In the young fish this difference between water-extract activity and that of the residue does not occur. It would seem, therefore, that in the case of the egg, solubility played an important part in the observed lipase activity; for instance, if the lipase were present only in the aqueous phase of the yolk then the protection of the glyceride-fat droplets against the action of the lipase until a late stage of the yolk-sac period would follow automatically since access of the enzyme to the glyceride-fat substrate would first require the relative exhaustion of the water-soluble lipoprotein of the yolk.

The morphology of the liver and yolk-sac circulation in the rainbow trout alevin has been studied by Portmann & Metzner (1929). Some 14 days after hatching the developing liver presses against the yolk-sac and increases in size as the yolk-sac diminishes. The area of contact on the anterior left aspect of the yolk-sac shows peculiar cytological changes which are initiated some 30 days after hatching. The double layer of splanchnopleur and the cell wall of the liver in contact with the yolk-sac syncytium disappear, so that the nuclei of the liver cells may be observed surrounded in part by their proper cytoplasm and in part by tissue of the syncytium of the yolk-sac wall.

Portmann & Metzner (1929) observe the development some 40 days after hatching of a vein which returns blood from the liver directly to the heart thus by-passing the yolk-sac circulation. This condition it is tempting to correlate with the newly established relationship between yolk-sac and liver. This fusion between liver tissue and yolk-sac wall is found well developed in sections of rainbow trout

material which had been reared in Cambridge for a total of 74 days at 10° C. In the case of Portmann & Metzner's (1929) material the initiation of fusion probably began at about the 70th day. Their material was, however, incubated at a lower mean temperature.

It seems probable, therefore, that this fusion of liver cells with the yolk-sac wall and the final absorption of the glyceride-fat from the yolk-sac may be correlated with the gross changes of metabolic activity initiated about the 66th day of incubation at 10° C. which has been described in a previously published paper (Smith, 1947). Whatever the mechanism may be for the accumulation of glyceride-fat during a considerable part of the yolk-sac phase, the increase in amount of glyceride fat and its later consumption in a relatively intense burst of heat production cannot be disputed.

(*c*) *The carbohydrates of the developing salmonid*

A direct comparison between the observations under discussion and the work of Hayes & Hollett (1940) on the Atlantic salmon is not easy, since they measured total free carbohydrate as glucose and as glycogen after water extraction and did not estimate that fraction of the carbohydrate attached to yolk protein. They report no glycogen in the salmon egg and only very small amounts of glucose (0·049 g./100 g. of egg), whereas in the trout, after hydrolysis, the total carbohydrate expressed as glucose is as much as 0·18 g./100 g. of egg. The discrepancy between these results seems to imply that most of the carbohydrate of the egg is bound to protein.

It is the decline in this bound carbohydrate that is presumably responsible for the first peak of carbohydrate combustion which was accordingly not detected by Hayes & Hollett, but has since been observed by Daniel (1947). The maximum carbohydrate content recorded by Hayes & Hollett was of the order of 0·6 g./100 g. of embryo and is half as much again as the value of 0·4 g./100 g. of embryo observed for the trout. The lower values here recorded might have arisen from loss of blood while preparing the embryo for analysis. The Canadian authors report glucose consumption during hatching, and at the same time an interruption in carbohydrate synthesis. Glycogen accumulates in the liver at a steady rate beginning some 30 days after hatching. They also report a significant combustion of glycogen and glucose initiated approximately 60 days after hatching; this would correspond to the combustion observed around the 66th day in the present series of observations during the initial phase of starvation.

Lafon (1947) reports a sugar content in *S. fario* and *S. irideus* material of the order of 2·0 mg./g. of fresh material. Hartmann *et al.* (1947), while publishing no specific figure for carbohydrate content, remark that on testing the egg contents with α-naphthol in concentrated sulphuric acid a violet colour, which indicates the presence of carbohydrate, develops only after 2 min. hydrolysis with 30% sulphuric acid. This would seem to support their conclusion 'auch die Kohlenhydrate liegen in gebundener Form vor'.

The glycogen of the developing salmon egg has been studied both by histochemical methods and by analysis by Daniel (1947), who finds a considerable glycogen content of 0·056 mg. per egg 5 days after laying which is distributed

throughout both blastoderm and yolk. Six days later the glycogen content has fallen to 0·026 mg. per egg. The temperature of incubation is unfortunately not known, even approximately, but since the eggs hatched after 49 days of development it must be assumed that they developed rather more slowly than the rainbow trout eggs under discussion. Daniel's results would therefore suggest glycogen consumption actually occurring during gastrulation overgrowth.

This present study was undertaken to secure evidence that might be expected to confirm unequivocally whether glycogen or other carbohydrate was consumed during gastrulation. The times of sampling were planned so as to show such a fall in the content of total carbohydrate, samples being analysed immediately before gastrulation began and as soon as gastrulation finished. Yet it was only after gastrulation was complete that a significant fall in total carbohydrate took place. So it must be concluded that the total glycogen consumed during gastrulation is not sufficient to reveal a change in total carbohydrate content by the methods of analysis here employed.

Several points in Daniel's paper are difficult to interpret. The wet weights of the embryo blastoderm show a peculiar history. From an average wet weight of 47 mg. 23 days before hatching the average falls in 8 days to 33 mg. and after a further 9 days it is no more than 34 mg. Eleven days later (2 days after hatching) the average wet weight is still only 53 mg.; after the passage of a further 9 days the wet weight is 45 mg. These figures can only be the result of sampling errors which are very high. While embryonic growth is restricted up to hatching there is no such restriction after hatching, so that the report of an embryo blastoderm slightly smaller 11 days after hatching than it was 23 days before hatching suggests an abnormal sampling error. Daniel also reports the existence of 'yolk-sac cells' within the syncytial wall. Unless salmon eggs differ significantly from those of trout these 'cells' can only be the emulsified droplets of yolk in process of digestion. Fixation and sectioning for the histochemical determination of glycogen is probably not sufficiently good for any precise interpretation; however, a careful examination of sections of comparable stages of the trout shows droplets as already reported but not any trace of nucleus in the drop as there should be if they are to merit the description 'yolk-sac cell'. Daniel also refers to his failure to confirm Portmann & Metzner's (1929) observation of fusion of liver cells with the yolk-sac syncytium. This may be because his series of samples break off too soon. His failure to observe the short burst of carbohydrate consumption when the change-over occurs in the type of fats catabolized might be attributed to the same cause; on the other hand, this period is brief and the complete cycle of combustion and replacement may have occurred between two of his later samples.

(*d*) *The sequence of energy sources in embryonic development*

The concept that energy sources for embryonic development form an ordered sequence of carbohydrate, protein and fat was developed by Needham (1931) in his *Chemical Embryology*, sect. 7.7; and further evidence in support of this view is briefly considered by him in sect. 3.37 of his *Biochemistry and Morphogenesis* (1942).

In the case of the rainbow trout the analysis of the daily heat production into portions relating to the particular fuel component consumed (Tables 5 and 6) makes it clear that a sequence of peaks of combustion in the use of carbohydrate protein and fat cannot be established for the trout. The metabolic substrates used before hatching cannot be identified with certainty; it has, however, been shown that a fall in the level of carbohydrate is delayed until after the 9th day of incubation at 10° C., by which time gastrulation is almost complete under these conditions of incubation. A striking feature of the main growth period, from the 39th to 60th days, is the relatively constant rate of heat production per gram of embryo tissue which may be attributed to the simultaneous combustion of protein and phosphatide fats. At the end of this phase there is a considerable though short-lived increase in the rate of heat production as glyceride-fat is burnt. Thus, if the energy sources of the sequence be modified to read, first, protein plus phosphatide fat, and secondly, glyceride-fat, these two substrates may be said in a general sense to have successive peaks of maximal consumption.

The consumption of carbohydrate in the earliest phase of development in teleosts could be inferred from the observations of Schlenk (1933), who studied the oxygen and carbon dioxide exchanges of developing trout eggs from fertilization until the 43rd day of incubation during continuous perfusion in a closed circulatory system. He was somewhat unusually successful both in the fertility of his material and in the complete absence of any mortality. From 36 hr. until the 9th day the respiratory quotient was of the order of 1·0, indicating carbohydrate consumption; subsequently values were of the order of 0·7. The glycogen estimations and histochemical study of salmon eggs by Daniel (1947) show that there is a significant consumption of glycogen between the 5th and 11th days of incubation.

The respiratory exchanges of *Fundulus* eggs were studied by Amberson & Armstrong (1933). They observed on the first day of incubation, out of a total period of 12 days to hatching, a respiratory quotient of 1·0. Fat consumption appeared to dominate between the 5th and 7th days. In the egg of *Fundulus*, which has a moderate supply of yolk, Phillips (1940) found the oxygen consumption was from the outset proportionate to the increase in cell number and to the amount of material in the embryonic mass. When gastrulation begins the slope of the curve of oxygen consumption with time changes sharply, but thereafter continues a steady rise. Respiration was cyanide-sensitive, showing maximum inhibition after 1 hr. of treatment. Pregastrular stages showed not a trace of inhibition of development until after 12 hr. of treatment with the same concentration of sodium cyanide. Corresponding stages of pelagic teleost eggs, which have very small food reserves, when similarly treated show marked inhibition of both respiration and development after only 20–30 min. exposure to sodium cyanide. Phillips concluded accordingly that, at least in pregastrular stages, development of the teleost egg was at the expense of a substrate which could be consumed under anaerobic conditions, but ultimately development depended upon a substrate operating through the cyanide-sensitive system. This interpretation raises the problem of the intermediary metabolism of the developing teleost. It is almost certain that the final

stages of metabolism in the embryo fish are concerned in the main with carbohydrate, small though the total amount may be. Changes in gross amount of total carbohydrate can be interpreted as a partial breakdown in supplies produced by some slowing of the synthesis of carbohydrate from other sources; and these changes can be correlated as we have seen above with certain periods of difficulty in the life of the developing fish.

Nothing in the present study has been done towards discerning the course of intermediary metabolism in embryonic development of the teleost. That remains a field for future study.

SUMMARY

1. The total carbohydrate content of the trout egg and embryo has been measured. The carbohydrate content falls during three periods: (i) the establishment of the blood circulation; (ii) hatching; and (iii) the onset of starvation.

2. The fats extracted by a non-polar solvent (carbon tetrachloride) from egg, embryo and yolk samples show no significant catabolism before hatching. These fats are considered to be glyceride-fats. In the yolk-sac stage such fats are only consumed between the 63rd and 80th days of incubation at 10° C.

3. The combustion of phosphatide fat deduced from the heat-production figures is conspicuous during the early stages of yolk-sac absorption, and may be correlated with the consumption of protein as an energy source.

4. These findings may be correlated with histological changes in the yolk-sac wall, and in the relation between yolk-sac and liver. The concept of a sequence of energy sources in ontogenesis is discussed.

REFERENCES

Amberson, W. R. & Armstrong, P. B. (1933). The respiratory metabolism of *Fundulus heteroclitus* during embryonic development. *J. Cell. Comp. Physiol.* **2**, 381–97.

Baker, J. R. (1944). The structure and chemical composition of the Golgi element. *Quart. J. Micr. Sci.* **85**, 1–71.

Daniel, R. J. (1947). Distribution of glycogen in the developing salmon (*Salmo salar* L.). *J. Exp. Biol.* **24**, 123–44.

Danielli, J. F. & Harvey, E. N. (1934). The tension at the surface of mackerel egg oil, with remarks on the nature of the cell surface. *J. Cell. Comp. Physiol.* **5**, 483–94.

Falk, K. G., Noyes, H. M. & Lauberblatt, I. (1927). Studies on enzyme action. XLV. Lipase actions of the whole trout at different ages. *J. Gen. Physiol.* **10**, 837–44.

Fauré-Fremiet, E. & Garrault, H. (1922). Étude des substances grasses et lipoïdes de l'œuf de Truite (*Trutta fario*). *Bull. Soc. Chim. Biol., Paris*, **4**, 379–87.

Halpern, G. R. (1945). Extraction of phospholipids in salmon roe. *Nature, Lond.*, **155**, 110.

Hartmann, M., Medem, F. Graf., Kuhn, R. & Bielig, H.-J. (1947). Untersuchungen über die Befruchtungsstoffe der Regenbogenforelle. *Z. Naturf.* **26**, 330–49.

Harvey, E. N. & Shapiro, H. (1934). The interfacial tension between oil and protoplasm within living cells. *J. Cell. Comp. Physiol.* **5**, 255–67.

Hayes, F. R. (1930). The metabolism of developing salmon eggs. (1) The significance of hatching and the role of water in development. *Biochem. J.* **24**, 723–34.

Hayes, F. R. (1930). The metabolism of developing salmon eggs. (2) Chemical changes during development. *Biochem. J.* **24**, 735–45.

Hayes, F. R. & Hollett, A. (1940). The carbohydrate metabolism of developing salmon eggs. *Canad. J. Res.* **18**, Sect. D, 53–65.

Hayes, F. R. & Ross, D. M. (1936). A quantitative study of the total fat content of developing salmon eggs. *Proc. Roy. Soc.* B, **121**, 358–75.

KUMAGAWA, M. & SUTO, K. (1908). Ein neues Verfahren zur quantitativen Bestimmung des Fettes und der unverseifbaren Substanzen in tierischem Materiel nebst der Kritik einiger gebräuchlichen Methoden. *Bioch. Z.* **8**, 212–347.

LAFON, M. (1947). Recherches sur l'utilisation des réserves vitellines au cours de l'embryogénèse. (1) Données sur l'embryon de Truite (*Salmo fario* et *Salmo irideus*). *Arch. int. Physiol.* **55**, 123–52.

MCCLENDON, J. F. (1915). On the formation of fats from proteins in the eggs of fish and amphibians. *J. Biol. Chem.* **21**, 269–74.

NEEDHAM, J. (1931). *Chemical Embryology*. Cambridge University Press.

NEEDHAM, J. (1942). *Biochemistry and Morphogenesis*. Cambridge University Press.

PHILLIPS, F. S. (1940). Oxygen consumption and its inhibition in the development of *Fundulus* and various pelagic fish eggs. *Biol. Bull. Woods Hole*, **78**, 256–74.

PORTMANN, A. & METZNER, G. (1929). Die Verbindung von Leber und Dottersack bei Teleostierlarven. *Verb. naturf. Ges. Basel.* **40**, 271–9.

SCHLENK, W. (1933). Kinetik der Oxydationsvorgänge in befruchteten Eiern. I. Mitteilung: Geschwindigkeit der Oxydation während der Embryonalentwicklung von *Trutta iridea* (Regenbogenforelle). *Biochem. Z.* **267**, 424–37.

SMITH, S. (1947). Studies in the development of the rainbow trout (*Salmo irideus*). 1. The heat production and nitrogenous excretion. *J. Exp. Biol.* **23**, 357–78.

TANGL, F. & FARKAS, K. (1904). Beiträge zur Energetik der Ontogenese. 4. Über den Stoff und Energieumsatz im bebrüteten Forellenei. *Pflüg. Arch. ges. Physiol.* **104**, 624–38.

TSAI, C. (1933). The total carbohydrate content of the liver tissue in the fasting rabbit: a method of estimation. *Chin. J. Physiol.* **7**, 91–102.

THE SIZE OF OMMATIDIA IN APPOSITION EYES

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(With Three Text-figures)

INTRODUCTION

The vertebrate eye appears to have an acuity that closely approaches a theoretical limit set by the size of the pupil. The optimum resolving power obtainable with a lens of diameter δ is given by the formula

$$\theta = 1{\cdot}22\ \lambda/\delta,$$

where λ is the wave-length of light and θ is the angle (radians) subtended by two point sources which can just be detected as double. In the case of the human eye, for example, the diameter of the pupil in bright daylight is about 0·3 cm., and the light to which it is most sensitive has a wave-length $5{\cdot}6 \times 10^{-5}$ cm. This gives an optimum resolving power of 47 sec., whereas the best observed two-point acuity in the human eye is just under 1 min. The figure was known empirically before it was realized that it approached a theoretical limit set by the physical properties of light, but it is interesting to realize in retrospect that the limit deduced from the pupil diameter would have been a good guide to the actual performance.

It is usually supposed that the resolving power of a compound eye is limited simply by the angle between neighbouring ommatidia, but it is possible that the small diameter of each ommatidium compared with the wave-length of light is really the limiting factor. If it were, then it would be most inefficient simply to scale up a small compound eye to suit a large insect; in this paper the optimum relation between the size of the eye and the size of the ommatidium is deduced and eyes of varying size have been measured to see if the relationship holds.

Johannes Müller (1826—quoted by Exner, 1891) suggested that each ommatidium of a compound eye of the apposition type was only sensitive to light coming from a point lying on, or close to, the axis of the ommatidium. This was not accepted immediately, because it was thought that each single ommatidium might have some ability to discriminate the direction of the light falling on it. Exner's examination of the anatomy and optics of compound eyes made the alternatives to Müller's suggestion unlikely, and Hecht & Wolf (1929) have shown that the optimum resolving power of the bee's eye corresponds to the smallest inter-ommatidial angle. Hassenstein has recently provided convincing evidence that the ommatidia behave as functionally independent units in an eye of the apposition type.

Model compound apposition eye

Müller's suggestion seems to be well established, and the potentialities of this type of eye are most easily discussed in terms of a model. The model is not supposed

to correspond accurately to the anatomical details of any particular eye, but it is supposed to imitate the optical arrangement. It consists of a number of directionally sensitive elements with a small angle between each of them, and together covering the required field of view. The acuity of such a model eye would depend on the angle between neighbouring elements, and at first sight it might be thought that the acuity could be improved indefinitely simply by reducing this angle and increasing the number of elements. This would only increase the acuity up to a certain point; beyond this point the acuity would be limited by the resolving power of each ommatidium, for it can easily be seen that acuity would no longer be improved when neighbouring ommatidia were set at such a narrow angle that overlapping occurred in the regions of the field from which they received light.

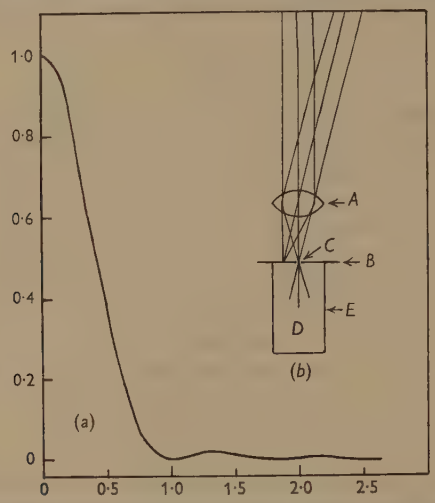

Fig. 1. (*a*) Relative effectiveness of light entering the ommatidium at increasing angles to its axis. Ordinates: effectiveness relative to effectiveness on main axis; abscissae: angle with main axis as multiple of minimum resolvable angle (θ). (*b*) Model of ommatidium. *A*, lens (cornea and crystalline cone); *B*, black screen (primary iris pigment); *C*, pinhole (point of contact of rhabdome and crystalline cone); *D*, photosensitive pigment; *E*, secondary iris pigment.

The usually accepted criterion for the resolving power of a telescope is the angle subtended by two point sources when the peak of the Airy disk of one falls at the first minimum of the other. In the case of an ommatidium functioning optimally, this corresponds to the smallest angle between two point sources such that one stimulates it to the greatest possible extent, and the other not at all. The resolving power according to this criterion is then given by the ordinary formula for a telescope:

$$\theta = 1{\cdot}22\,\lambda/\delta,$$

θ = resolving power, λ = wave-length of light, δ = diameter of ommatidium.

Fig. 1 (*a*) shows how the amount of light absorbed by such an ommatidium would vary with the angle the incident light made with the main axis of the ommatidium.

(*b*) shows a possible structure for achieving this resolution, and the actual anatomy appears to correspond to this proposed model. One would suppose that improvements in acuity would have survival value for many classes of insect; let us therefore suppose that this theoretical limit is achieved, and see what generalizations about the structure and performance of compound eyes follow.

First consider what happens if ommatidia of resolving power θ are set at different angles ϕ to each other. Two point sources will be resolved if they can each stimulate an ommatidium, leaving an unstimulated one in between. Fig. 2 shows the amount of light reaching the central ommatidium as a fraction of the light reaching each of the outer ommatidia, as ϕ, the angle of separation of the ommatidia, is reduced; it is supposed that the two point sources lie on the main axes of the two flanking ommatidia.

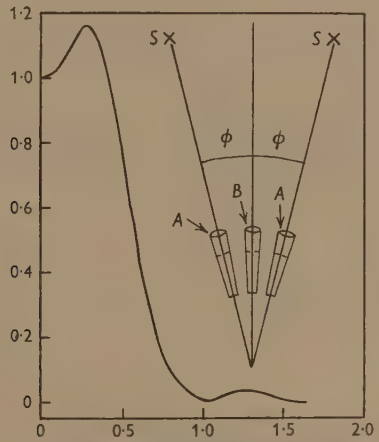

Fig. 2. Three ommatidia (A, B, A), of limiting resolving power θ, are set at an angle ϕ, and illuminated by two sources (SS) on the axes of AA. The graph shows illumination of B relative to A (ordinates) as ϕ/θ (abscissae) is increased.

It will be seen that when $\phi \geqslant \theta$ the two point sources excite the central ommatidium little or not at all. In a compound eye in which the angular separation of the ommatidia is equal to, or greater than, the angular resolving power of each ommatidium, there should be no difficulty in resolving points separated by twice this angle. On the other hand, when $\phi = 0{\cdot}4\theta$, the central ommatidium is actually receiving more light than the flanking ommatidia, and the two points would not be resolved. One would therefore suppose that such an eye, in which the angular separation of the ommatidia was less than 0·4 of the angular resolving power of the ommatidia, would fail to resolve points separated by twice the inter-ommatidial angle; there would seem to be no point in constructing an eye like this.

The first conclusion reached is, therefore, that ϕ should be greater than $0{\cdot}4\theta$ in a compound eye in which ϕ is the angular separation of the ommatidia, and θ is the angular resolving power of each ommatidium. If the assumption that the theoretical

resolving power is closely approached is granted, one would expect $0{\cdot}4\theta < \phi < \theta$ in those types of insects in which acuity has survival value.

Imagine the problems concerned in designing an eye for an insect. It must obviously be of limited size, and it must presumably cover a wide field of view. The first question would be 'how large should the ommatidia be?' If they are too small, each one will have a poor resolving power, and the acuity of the whole eye could be improved by having fewer, larger, ommatidia. If they are too big, the angle between them must be large, and the acuity could be improved by having more, smaller, ones and reducing the angle between them. For an eye of good acuity the actual size should be smaller than the size which would make acuity be limited by inter-ommatidial angle, and larger than the size which would make acuity be limited by the resolution of each ommatidium. These two limits can be worked out as follows:

δ = Diameter of a single ommatidium (cm.).
ϕ = Angle between axes of ommatidia (radians).
n = Number of ommatidia in a row.
d = Length of a row (cm.).
a = Angular field of view of a row (radians).
θ = Resolving power of an ommatidium (radians).
λ = Wave-length of light (cm.).

From $n\phi = a$ and $n\delta = d$,

$$\phi = \frac{a\delta}{d}.$$

From the formula for the resolving power of telescope

$$\theta = \frac{1{\cdot}22\lambda}{\delta}.$$

Upper limit, $\phi = \theta$,

$$\delta = \sqrt{\frac{1{\cdot}22\lambda d}{a}}.$$

Lower limit, $\phi = 0{\cdot}4\theta$,

$$\delta = \sqrt{\frac{0{\cdot}49\lambda d}{a}}.$$

Real compound eyes

There are now two points which can be checked against the actual anatomy of a compound eye. First, in any eye, one can see if the relationships between inter-ommatidial angle, ommatidial size, and total size of eye, fall within the limits suggested. Secondly, one can see if ommatidial size varies in the predicted way in a range of eyes of different size. The first point can be decided by measuring the diagrams in Baumgärtner's (1928) paper on the vision of bees. The ommatidia were taken in groups of six or four from his figs. 31 and 32, and their breadth measured at right angles to their long axis at the level of the cornea. His figures for inter-ommatidial angle were used.

In the centre of the eye, where the inter-ommatidial angle is least, the ommatidia have a mean diameter of $21{\cdot}6\mu$. Taking $\lambda = 5 \times 10^{-5}$ cm. (Sander 1933), $\theta = 1{\cdot}6°$. The smallest inter-ommatidial angles in the bee occur in this region, and have a

mean value of 0·97° over the region measured. This corresponds to $\phi = 0{\cdot}61\theta$. The largest angles, measured in a vertical plane, occur at the tops of the eye, and have a mean value of 1·84°. Over this region the mean diameter of the ommatidia is reduced to 16·9 μ, so that their resolving power is $\theta = 2{\cdot}07°$, and $\phi = 0{\cdot}89\theta$. In the lowest region of the eye ϕ is increased to 1·74°, but θ remains at 1·6°. The bee's eye is highly 'astigmatic' in the sense that the angle between ommatidia measured in a horizontal plane is greater than any of the figures given above. In the central region it averages 2·8°; the mean diameter of the ommatidia measured in this direction is 20·9 μ, so that $\phi = 2{\cdot}8° = 1{\cdot}67\theta$. At the edges the inter-ommatidial angles get bigger than 4°, so that $\phi > 2{\cdot}5\theta$.

The conclusion reached from these measurements was that the bee's eye is constructed in accordance with the principles that were put forward, but only in those regions where the acuity is greatest. Elsewhere the ommatidia are too large (or the inter-ommatidial angle too large) to give the eye optimum acuity for the space covered.

Before it was realized how much the inter-ommatidial angle varied in different parts of the same eye, the eyes of an assortment of museum specimens of insects were examined. A plane, usually vertical, was found in which the eye appeared, by inspection, to cover 180°. The overall 'height' of the eye in this plane was then measured. It was assumed that a section of the eye in this plane would be nearly semicircular, and that the distance measured would be the diameter of the semicircle. These observations and measurements gave an estimate of d and a. From these the limiting values of δ, the diameter of an ommatidium, for optimum resolution were calculated. The actual diameters of the ommatidia were measured for comparison. All these observations and measurements were made with a micrometer eyepiece in a dissecting microscope.

The agreement between the observed and the calculated sizes of ommatidia was only moderately good, and the plot of height of eye against diameter of ommatidium failed to show the expected square-root relationship; a straight line would have fitted equally well. It was clear, however, that this disappointing result did not simply represent deviation from optimal design. The eyes were of very different shapes; some, like the dragonfly's, were almost exactly hemispherical protuberances, whereas others, like the eye of the praying mantis, were moulded to the contour of the head. The spectral sensitivity curves of insect eyes are not necessarily alike, and the optimal ommatidial size might vary accordingly. It is also possible that insects living where the light is feeble might sacrifice acuity for sensitivity by having large ommatidia.

To reduce variation from these causes the eyes of twenty-seven Hymenoptera were examined. They were all diurnal species, and were chosen to cover as wide a range of eye size as possible, the largest being a tropical bee 60 mm. long with a 5 mm. eye, and the smallest a Chalcid less than 1 mm. long with an eye of 0·2 mm. A list of these insects is given at the end of this paper in order of increasing eye size. The external shape of the eyes was remarkably similar throughout the group, except for a tendency to be more globular in some of the smaller ones; these species are placed in brackets in the list.

Fig. 3 shows the diameters of the ommatidia plotted against the square root of the heights of the eyes. The point for the bee's eye is indicated by a cross. If all the eyes were the same shape, had the same distribution of acuity in the visual field, and the same ratio of ϕ/θ, then the points should lie on a straight line through the cross and the origin. There is a tendency for the small eyes to have rather bigger ommatidia than expected. This might be because small ommatidia can only collect little light, which would limit contrast sensitivity and necessitate a higher value of ϕ/θ, but, considering that no allowance has been made for factors such as the thickness of ommatidial walls, the deviations are small.

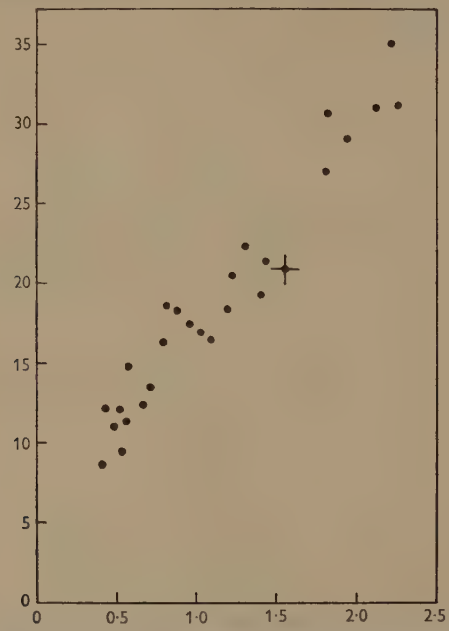

Fig. 3. Diameter of ommatidia in twenty-seven Hymenoptera.
Ordinates: diameter in μ; abscissae: square root of height of eye in mm.

This result supports the suggestion that the resolving power of the compound eye is limited by the diameter of the ommatidium in relation to the wave-length of the visible light. In any given eye, of course, the resolution is also limited by inter-ommatidial angle, but the way in which ommatidial size and inter-ommatidial angle are adjusted in eyes of different size suggests strongly that the wave structure of light is the limiting factor in the design of the compound eye.

DISCUSSION

The rather theoretical approach which has been presented gives a satisfactory explanation of the size of the ommatidia in eyes of different sizes, and one is tempted to make further generalizations about compound eyes. It was shown that the lower limit to the useful size of an ommatidium in an eye of length d and field a was given by

$$\delta = \sqrt{\frac{0{\cdot}49\lambda d}{a}}.$$

The minimum resolvable angle of the eye will be twice the inter-ommatidial angle ϕ, and this angle will be smallest when the ommatidia are smallest if the other dimensions of the eye are fixed. The minimum resolvable angle r of the compound eye will therefore be given by

$$r = 2\phi = 2\sqrt{\frac{0{\cdot}49\lambda a}{d}}.$$

In the case of a simple eye the minimum resolvable angle is inversely proportional to the pupil diameter. It is difficult to compare the merits of the two types of eye, because pupil diameter, in a simple eye, is obviously not a measure of the 'size' comparable with the length of a row of ommatidia d in a compound eye. Nevertheless, one can say that an increase of size allows greater improvement of acuity in simple eyes than in compound eyes.

Oddly enough one comes to the same conclusion in considering sensitivity. This must depend on many factors outside the scope of this paper, such as the quantity of photosensitive pigment, the percentage of incident light it absorbs, and its stability, but the actual amount of light admitted by the optical system is clearly one of the most important factors. The human eye admits less than 1000 quanta per second from a point source which is just visible. The bee's eye would admit one quantum about every 3 min. from such a source, and to be constantly visible the light would certainly have to be increased in intensity more than a thousand times. Since in a compound eye of optimum acuity the area of an ommatidium is proportional to the length of the eye, the sensitivity might also be expected to increase directly in proportion to the length. In the simple eye, on the other hand, the area of the pupil increases as the square of its diameter, and an increase of size can bring a correspondingly greater improvement in sensitivity.

At first sight one is inclined to judge that the compound eye is an inefficient contraption compared with the simple eye of vertebrates. But it is not, relatively, so inefficient for small eyes, and the insect may perhaps have other tricks to humble the critical theorizing biologist; the bee's visible spectrum extends into the ultra-violet (Bertholf, 1931; Wigglesworth, 1950), and there is also evidence for a mechanism sensitive to the plane and intensity of polarization of light (von Frisch, 1950; Autrum & Stumpf, 1950). The additional information so obtained might easily compensate for the poor acuity of this type of eye, and where sensitivity is required the apposition eye has been elegantly modified to the superposition type.

List of insects measured in order of increasing eye size

Aphelinus tibialis; Dacnusa areolaris; (Aphidius ulmi); Dacnusa stramneipes; Tetrastichius brevicornis; (Praon lepelleyi); (Aphidius ervi); (Paxylomma buccata); (Polemochartus liparae; (Macrocentus thoracicus); Macrocentus marginator; (Dufourea halictula); Chelostoma campanularum; Prosopis hyalinata; Heriades truncorum; Symmorphus sinustissimus; Stelis phaeoptera; Stelis punctatissima; Eumenes coarctata; Ancistrocerus callosus; Apis mellifera; Vespula germanica; Bombus terrestis; Scolia speciosa; Vespa crabro; Scolia procer; Salius sycophanta.

Those placed in brackets had eyes of an unusual, more globular, shape.

SUMMARY

1. In a compound apposition eye the acuity may be limited by the inter-ommatidial angle or by the optical performance of each ommatidium. In the honey-bee the performance of the ommatidia must approach the theoretical limit set by diffraction if the whole eye resolves points separated by twice the inter-ommatidial angle.

2. The eyes of twenty-seven other species of Hymenoptera were measured, and the results show that in eyes of different sizes the number of ommatidia is adjusted so that inter-ommatidial angle is just below the limiting resolving power of the ommatidia; this is the condition for optimum acuity in a compound apposition eye.

3. When this condition is fulfilled the minimum resolvable angle is inversely proportional to the square root of the linear dimensions of the eye. Acuity increases with size more rapidly in the simple type of eye.

I am very grateful to Mr John Smart for allowing me to use specimens in the collections of the University Museum of Zoology, and for helping me in the selection of suitable species.

REFERENCES

Autrum, H. & Stumpf, H. (1950). Das Bienenauge als Analysator für polarisiertes Licht. *Z. Naturforschung*, **5***b*/**2**, 116–22.

Baumgärtner, H. (1928). Der Formensinn und die Sehschärfe der Bienen. *Z. vergl. Physiol.* **7**, 56–143.

Bertholf, L. (1931) The distribution of stimulative efficiency in the ultra-violet spectrum for the honey bee. *J. Agric. Res.* **43**, 703–713.

Exner, S. (1891). *Die Physiologie der Facettirten Augen.* Franz Deuticke.

von Frisch, K. (1950). *Bees; their Vision, Chemical Senses, and Language.* New York: Cornell University Press.

Hassenstein, B. (1951). Ommatidienraster und afferente Bewegungsintegration. *Z. vergl. Physiol.* **33**, 301–26.

Hecht, S. & Wolf, E. (1929). The visual acuity of the honey-bee. *J. Gen. Physiol.* **12**, 727–60.

Müller, J. (1826). *Zur vergleichenden Physiologie des Gesichtssinnes.* Leipzig.

Sander, W. (1933). Phototaktische Reaktionen der Bienen auf Lichten verchiedener Wellenlänge. *Z. vergl. Physiol.* **20**, 267–86.

Wigglesworth, V. B. (1950). *The Principles of Insect Physiology.* London: Methuen.

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